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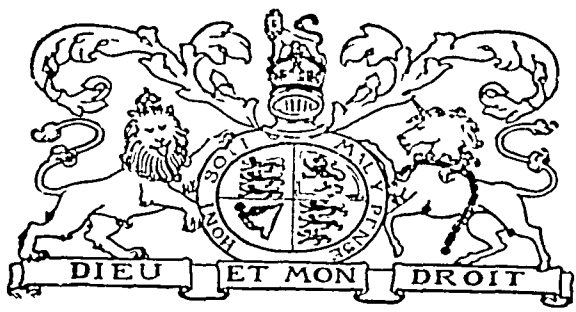
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THE INDIAN JOURNAL OF MEDICAL RESEARCH

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THE DIRECTOR-GENERAL INDIAN MEDICAL SERVICE

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STUDIES IN THE VALUE OF ETHERIZED SHEEP VACCINE IN THE PROPHYLACTIC TREATMENT OF RABIES

Part I

THE EFFECT OF ETHER ON 'FIXED VIRUS' IN INFECTED BRAINS OF SHEEP

BY

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AND

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[Received for publication, November 28, 1929]

CONSIDERABLE work has been done regarding the use in anti-rabic treatment of a vaccine in which the fixed virus has been attenuated by ether This vaccine is given in considerably bigger doses than the carbolized vaccine

With the possibility of the introduction into the Pasteur Institutes in India of treatment of patients with ether vaccine, one must consider the use of other suitable animals in addition to the rabbit Rabbits have been the animal of choice ever since Pasteur's work, but these are expensive and are not always easy to get in large numbers The Pasteur Institute at Calcutta uses the brains of lambs in the preparation of carbolized anti-rabic vaccine and the results of treatment obtained there are as good as those of other institutes in India The institutes at Coonoor and at Bombay occasionally supplement rabbits by using sheep

A series of experiments as to the value of ether vaccine prepared from sheep's brain infected with our stock of 'Fixed Virus' in the prophylactic treatment of rabies are in progress The effect of ether on Fixed Virus sheep's brain forms the subject matter of this communication

Experiment 1

A sheep was inoculated subdurally with the Fixed Virus in use at this Institute The animal developed rabies on the 6th day, was moribund on the 7th day and was killed the same day The brain of the animal was divided into four equal portions and one of these was suspended in ether for 72 hours and another for 84 hours Six rabbits were inoculated under the dura-mater with 0 002 gramme of the 72-hour ether brain and all of them showed definite signs of rabies on the 6th day The same number of rabbits were used for 84-hour ether brain with identically the same results The virus in this experiment was neither killed nor attenuated

Experiment 2

One-fourth of a Fixed Virus sheep's brain was taken and under sterile conditions was chopped up into fine pieces and then suspended in ether in fine mesh sterile mosquito-netting bags

(*a*) Six rabbits were inoculated subdurally with an emulsion of sheep's Fixed Virus brain that had been immersed in ether for 72 hours Three developed rabies on the 6th day, one on the 7th day and one on the 8th day The rabbit surviving 30 days after infection was subsequently inoculated with stock fixed virus and this developed rabies on the 6th day

(*b*) Six rabbits were inoculated subdurally with an emulsion of sheep's Fixed Virus brain that had been immersed in ether for 84 hours Three developed rabies on the 7th day and one on the 10th day The two rabbits surviving 30 days after infection were inoculated with stock fixed virus and these developed rabies on the 6th day, thus showing the absence of natural immunity

(*c*) Six rabbits were inoculated subdurally with emulsion of sheep brain after suspension in ether for 96 hours None developed rabies The animals were observed for 30 days

(*d*) Six rabbits were inoculated subdurally with emulsion of sheep brain after suspension for 120 hours in ether None developed rabies 30 days after infection

The dose used for testing infectivity in (*a*), (*b*), (*c*) and (*d*) was 0 002 gramme of brain substance When the infected brain was chopped into fine pieces and then suspended in ether, the fixed virus was attenuated after 72 hours and killed after 96 hours

Experiment 3

Ether carbolized Fixed Virus vaccine—Portions of fixed virus sheep's brain finely cut up were immersed in ether for periods of 72 and 84 hours in mosquito-netting bags and then emulsified in 1 per cent carbolized saline After 24 hours standing at room temperature the emulsion was filtered and an equal quantity of sterile saline added to bring the finished vaccine to a 1 per cent strength in 0 5 per cent carbolized saline

(*a*) Six rabbits were inoculated subdurally with 0 2 c c of 72-hour ether carbolized vaccine One developed rabies on the 7th day The remaining rabbits which showed no signs of rabies for 30 days after infection were subsequently inoculated subdurally with our stock fixed virus and all these developed rabies

(*b*) Six rabbits were inoculated subdurally with 0 2 c c of 84-hour ether carbolized fixed virus sheep vaccine One developed rabies on the 8th day The five survivors 30 days after infection were subsequently inoculated subdurally with stock fixed virus and all developed rabies

The addition of 1 per cent carbolic acid to 72- and 84-hour ether brains causes marked attenuation of the fixed virus

Experiment 4

Ether carbolized Fixed Virus vaccine —In this experiment, portions of fixed virus sheep brain finely cut up were immersed in ether for 72 hours and then emulsified in 1 per cent carbolized saline, incubated at 37°C for 24 hours, and subsequently filtered and saline added to bring the finished vaccine to a 1 per cent strength in 0 5 per cent carbolized saline

(*a*) Six rabbits were inoculated with 72-hour ether carbolized fixed virus sheep vaccine None developed rabies

(*b*) Six rabbits were inoculated with 84-hour ether carbolized fixed virus sheep vaccine None developed rabies

(*c*) Six rabbits were inoculated with 96-hour ether carbolized fixed virus sheep vaccine None developed rabies

(*d*) Six rabbits were inoculated with 108-hour ether carbolized fixed virus sheep vaccine None developed rabies

The animals were kept under observation for 30 days

The fixed virus is killed in 72-hour ether carbolized vaccine incubated for 24 hours at 37°C

Cornwall and Beer (1926) found that the fixed virus in use at the Institute in Coonoor was killed by immersion in ether for 48 hours They used half brains of fixed virus rabbit's brain

Alivisatos (1922) states that a 24 to 36 hours immersion of infected rabbit's brain in ether is practically without effect, a 48 to 96 hours immersion lengthens the incubation in rabbits by 10 to 18 days, after 120 hours in ether the brain was not always virulent and finally the virus after 140 hours immersion was completely killed He was working with a different strain of fixed virus

Cunningham, Nicholas and Lahiri (1927) found marked attenuation of the virus after immersion in ether for periods longer than 36 hours and no evidence has been obtained of the viability of the virus when the brain material has been immersed for periods longer than 84 hours

In our experiments using infected brains of sheep we find the fixed virus is much more resistant to ether than an infected rabbit's brain

SUMMARY

The resistance to ether of the fixed virus in use at the Pasteur Institute, Coonoor, has been tested by immersion of infected brains of sheep in the fluid, with the following results —

1 When the brain is cut into large pieces, 84-hour immersion has no effect

2 When cut into fine pieces and then suspended in ether, there is attenuation after 72 hours and death after 96 hours

3 The addition of 1 per cent carbolic acid to 72-hour ether brain and subsequent incubation at 37°C for 24 hours, kills the fixed virus

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THE ACTION OF OPIUM AND NARCOTINE IN MALARIA

BY

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(Drug Addiction Series No. 6.)

[Received for publication, December 4, 1929.]

IN the report of the Opium Commission of 1895, it was stated that the habit of taking opium prevails in excess among the population of low-lying, damp and malarious districts of India, and it was implied that this drug has an anti-malarial action. Dr. Roberts in his note said that the belief in the usefulness of opium in the complaints of damp and malarious districts was very widely spread. According to him the consumption of opium in the marshy districts of England was very large in the days when lands were undrained and malaria was prevalent. The evidence laid before the Opium Commission showed that in some districts of India the local consumption of opium bore a close relationship to the greater or less prevalence of malaria in the localities. In determining the question from a scientific point of view as to what extent opium has the power to cure and prevent genuine malarial fever, Dr. Roberts pointed out that the two important and abundant alkaloids occurring in opium are morphia and narcotine or anarcotine. Morphia represents the anodyne and hypnotic properties of the drug and narcotine is a bitter crystalline alkaloid resembling quinine and like that substance possesses tonic and anti-periodic properties. A perusal of Table I will show that in Patna or Behar opium the narcotine content is nearly double that of morphia content. In Malwa opium

narcotine is slightly larger in quantity than morphia, while in Smyrna opium the morphia content is more than 4 times that of narcotine

TABLE I

Description of opium	Morphine per cent	Narcotine per cent
Patna opium (Behar provision cake)	3·98	6·36
Malwa opium	4·61	5·14
Smyrna opium	8·27	1·91

OPIUM IN MALARIA

So far as the action of opium in malaria is concerned, it has been shown in a previous paper that this drug is not much used at the present time, as a household remedy for its supposed prophylactic or curative effects In some of the low-lying districts of the Punjab along the course of such rivers as the Jhelum, the Chenab and the Indus, the climate is very damp and a virulent type of malaria prevails The spleen index in these areas is also very high but the consumption of opium is very small indeed, while in some of the comparatively dry and healthier areas the consumption is enormous We have made careful inquiries in these areas but we have not discovered the existence of any belief among the rural or urban population in the anti-malarial properties of opium in combating an attack or in preventing recurrences There is no doubt that the main factor responsible for the extent to which the drug was used was the availability of opium in a particular locality When opium was grown in these very areas, its consumption was much greater than it is at the present time

Opium on account of its sedative effects undoubtedly ameliorates the symptoms produced by malaria, but it has no curative action whatsoever in this disease Our everyday experience among opium addicts in the central districts of the Punjab convinced us that they suffered just as much from malaria as those who were not addicted to the drug, during the seasons when this disease was prevalent Opium has neither a prophylactic nor a curative action in the disease.

NARCOTINE IN MALARIA

As regards the suggestion made by Dr Roberts that narcotine may possibly be the alkaloid which may have anti-malarial properties, this belief

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ten days he received 84 grains of the alkaloid Table II gives the details regarding this patient

TABLE II

Date	Treatment	Temperature (Degrees Fahrenheit)		Number of parasites in blood, per c mm	Remarks
		Maximum	Minimum		
16-7-29		99·0	98·0		
17-7-29	Narcotine gr iii, b d.s	103·0	97·0	*P malariæ* present in the blood	
18-7-29	Do	103 8	97·0		
19-7-29	Do	98·0	97 0		
20-7-29	Do	102·0	97 6		
21-7-29	Do	100 6	97 0		
22-7-29	Narcotine gr iii, t d s	98·0	97·0	Trophozoites 530 per c mm Gametocytes 33 per c mm	
23-7-29	Do	104 4	96 8	700	
24-7-29	Narcotine gr iii, 4 times daily	102·8	99·6	50	
25-7-29	Do	102·0	99·2	45	
26-7-29	Do	100·0	98·6	367	
27-7-29	Mist quinine sulph, ℥i, t d.s	103 0	99 0	150	
28-7-29	Do	98·0	97 0	No parasites seen	
29-7-29	Do	97 0	96·0		
		Temperature remained normal			

It will be observed that even 12 grains of narcotine per day had no effect whatsoever on the clinical symptoms of the patient The number of parasites varied but they still persisted in the blood The patient tolerated the drug well and it appeared to ameliorate the symptoms and discomfort produced by the disease As soon as the patient was put on quinine, the temperature came down and the parasites disappeared from the peripheral blood

Case 2.—The patient was a female child 8 years old, suffering from benign tertian infection, trophozoites (*P vivax*) were found in the peripheral blood

Details of the condition and the number of parasites present in the blood are given in Table III

TABLE III

Date	Treatment	Temperature (Degrees Fahrenheit)		Number of parasites in blood per c mm	Remarks
		Maximum	Minimum		
1-8-29		103·0	100·0	950 (*P vivax*)	
2-8-29	Narcotine gr ii, t d s	103·0	100·0	1,000	
3-8-29	Do	101 5	99·2	628	
4-8-29	Do	99 8	98·4		
5-8-29	Do	100·4	98·4	320	
6-8-29	Do	99 4	99·0	556	
7-8-29	Do	101 0	98 4	1 350	
8-8-29	Do	100·8	98 4	2,200	
9-8-29	Do	101 5	98 4	2,300	
10-8-29	Do	102·4	98·4	1 040	
11-8-29	Do	101 2	98 4		
12-8-29	Mist quinine sulph ℥ss t d s	102·4	98·4	1 600	
13-8-29	Do	99·0	98·0	Parasites disappeared from the peripheral blood	

It will be seen that narcotine in doses of two grains three times a day administered for a period of ten days had no effect on the clinical symptoms of the disease The patient was getting rigors and was becoming anæmic The number of parasites present per c mm of blood increased to more than double the number originally present while narcotine was being administered Quinine was given on the 11th day after administration and the temperature of the patient became normal immediately and the parasites disappeared from the peripheral blood and other clinical symptoms abated

Case 3—This patient was a female child aged 8 years who suffered from a mixed infection of benign and malignant tertian fever Ring forms of *P vivax* and *P falciparum* were both found in the peripheral blood A perusal of Table IV shows that narcotine in doses of two grains three times a day had no effect on the temperature and the number of parasites actually increased As soon as quinine was administered the temperature came down to normal

TABLE IV

Date	Treatment	Temperature (Degrees Fahrenheit)		Total count of parasites in blood per c mm	
		Maximum	Minimum		
1-8-29		105·0	98·0	*P falciparum*	3,279
2-8-29	Narcotine gr ii, t d.s	103·0	98·0	*P vivax* *P falciparum*	1,733 667
3-8-29	Mist quinine sulph, ℥ss, b d	104·0	98·4	*P vivax* *P falciparum*	1,308 3,077
4-8-29	Do	99·0	98·0	No parasites seen	

Case 4.—This patient was suffering from malignant tertian infection and *P falciparum* trophozoites and gametocytes were found in the peripheral blood. He was given 3 grains of narcotine 3 times a day for 3 days and for two succeeding days 3 grains four times a day. A perusal of Table V will show that even 12 grains of the alkaloid daily had no effect on the clinical symptoms of the patient. The number of parasites present per c mm of blood remained practically the same after 51 grains of narcotine has been given, 10 grains of quinine sulphate given twice daily brought down the temperature immediately and the parasites disappeared from the peripheral blood.

TABLE V

Date	Treatment	Temperature (Degrees Fahrenheit)		Total count of parasites in blood per c mm	
		Maximum	Minimum		
22-7-29	Narcotine gr iii, t d.s	100·0	98·4	Trophozoites Gametocytes	3,667 66
23-7-29	Do	99·8	97·4	Parasites	40
24-7-29	Do	99·5	97·4	Do	971
25-7-29	Narcotine gr iii, 4 times	99·0	97·5	Do	100
26-7-29	Do	99·4	98·0	Do	600
27-7-29	Mist quinine sulph, ℥i, b d	99·8	98·0	Do	3,200
28-7-29	Do	99·0	97·0	No parasites seen	
		Temperature remained normal			

Case 5—This patient suffered from a mixed malignant tertian and benign tertian infection and before his admission to hospital was having rigors and fever every alternate day Trophozoites of *P falciparum* and *P vivax* were found in the peripheral blood Narcotine in doses of three grains three times a day was given for 2 days The dose was then increased four grains four times a day for two more days Although the patient was not showing any symptoms of the disease, the malarial parasites present in the blood were not affected so far as their number was concerned Some parasites appeared to show degeneration but this was probably due to the fact that the patient was undergoing a spontaneous cure, and not due to any effect on the part of narcotine

TABLE VI

Date	Treatment	Temperature (Degrees Fahrenheit)		Total count of parasites in blood per c mm	
		Maximum	Minimum		
22-7-29		99·2	97·0	*P vivax* *P falciparum*	133 267
23-7-29	Narcotine gr iii, t d.s	98·0	97·4	*P vivax*	200
24-7-29	Do	98·0	97 0	Do	100
25-7-29	Narcotine gr iv, 4 times a day	98·6	97 0	Do	99
26-7-29	Do	98·4	97 5	Do	50
27-7-29	Mist quinine sulph, ℥i, t d.s	88·0	97·0	Do No parasites seen	450

Case 6—This patient was suffering from malignant tertian infection and showed *P falciparum* rings Narcotine 3 grains was given three times a day but had little effect on the number of parasites present in the peripheral blood The temperature came down in this patient the day after narcotine was administered, but the parasites were found in the blood daily even after 45 grains of narcotine had been administered Administration of quinine produced immediate effect and the parasites disappeared The details of this patient are found in Table VII

TABLE VII

Date	Treatment	Temperature (Degrees Fahrenheit)		Total count of parasites in blood per c mm
		Maximum	Minimum	
1-8-29		99·4	98·4	*P falciparum* 314
2-8-29	Narcotine gr ii, b d.s	102·8	98·6	
3-8-29	Do do t d.s	99·8	98·4	100
4-8-29	Do do do	98·4	98·0	
5-8-29	Do do do	99·0	98·4	None seen
6-8-29	Do do do	98·8	98·4	*P falciparum* 50
7-8-29	Omit narcotine	99·0	97·4	40
8-8-29		99·2	98·0	250
9-8-29	Mist quinine sulph ℈i b d	98·4	97·0	
10-8-29		99·0	98·0	None seen
		Temperature remained normal		

DISCUSSION OF RESULTS

A study of the above cases will show that narcotine even in large doses had no effect whatever on the parasites of *P malariæ, P vivax* or *P falciparum* circulating in the peripheral blood The microscopic appearance of the parasites did not show any changes and the virility of the parasites remained unaltered inasmuch as they grew quite well in cultures made from the blood of patients under narcotine treatment The administration of quinine produced a remarkable effect in these cases, the parasites disappeared immediately after the drug was given and the temperature came down to normal Even such large doses of narcotine as 4 grains 4 times a day produced no untoward effects on the patients On the other hand the symptoms of the disease appeared to be ameliorated and the patients looked more comfortable under the influence of the alkaloid even when they were running a temperature

Besides the series of cases given above in which the effects of drug were thoroughly observed with counts of parasites and cultures, two other small series were tried, one by Major Kernahan, I M S , in Peshawar and the other by Dr Belgard in Bengal It is not necessary to give details of these series but in none of them the drug showed any marked curative properties in this disease

Conclusions

(1) There is no evidence to show that opium has any prophylactic or curative effect in malaria

(2) The alkaloid narcotine even in such large doses as 10 to 15 grains daily has no effect on the parasites of any forms of malaria circulating in the peripheral blood The temperature of the patient remains unaffected and rigors and paroxysms continue

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THE ACTION OF OPIUM IN DIABETES

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IT IS a common belief among the people in India that small doses of opium produce beneficial effects in those suffering from diabetes In Bengal where glycosuria is frequently met with among the well-to-do classes and also in other parts of India, it is not uncommon to meet people who take small doses of this drug with apparent improvement in the clinical symptoms which accompany this condition, e g, polyuria, thirst, etc The sugar present in the urine is also reduced and may entirely disappear As we were unable to find in the literature any record of systematic investigation of the effects of small doses of opium on the blood-sugar and urine-sugar of diabetics, we decided to carry out observation on a series of our patients in the Carmichael Hospital for Tropical Diseases

The patients suffering from this disease were put on a balanced diet so that the excretion of sugar in the urine was more or less uniform The study of the influence of any drug on diabetes cannot be made without the full realization of the fact that the results are only of value if we know what the food intake is and what effect the quantity of carbohydrates allowed produces on the blood and urine-sugar The patients were, therefore, kept strictly on the same diet, beginning a few days before the drug under trial was tested and carried on for a few days after the drug was stopped All the patients were put on a strict diet of known value and some little time was allowed before the administration of the drug, to get the daily output of sugar in urine to run to a constant level The drug was then given in gradually increasing doses, the total daily output of urine and of sugar excreted were carefully measured every day and the blood-sugar examined at regular intervals The patients were also regularly weighed Great care was taken to maintain the same standard

diet throughout the test and also to the proper control period before and after the administration of the drug Opium was administered in form of a mixture so constituted that one ounce contained 1 grain of the drug The taste and smell of opium were effectively concealed by putting in oil of citronella so that the patient had no idea as to the nature of the drug he was taking This was done to exclude the psychic element The initial dose was 1 to 2 grains a day and this was increased every 3rd or 4th day by 1 grain till about 6 to 8 grains were taken daily In some cases 10 to 12 grains were given in this way without the least discomfort to the patient The drug was then stopped either suddenly or by a gradual reduction of the dosage

Case 1—J P D, Hindu male, aged 35 years, admitted to hospital, suffering from mild type of diabetes Father suffered from the disease Sugar first noticed in the urine a month ago, now complains of thirst but no marked polyuria or increased frequency of micturition Habits sedentary, looks somewhat stout but appearance not unhealthy Opium treatment from 21st August, 1928 to 30th August, 1928, total quantity given 22 grains

TABLE I

Date	Urine			Blood-sugar per cent	Body-weight in lb	Opium administered daily in grains
	Total quantity in c c	Sugar per cent	Total sugar in grms			
16-8-28	2,040	2·0	40·8	0·230	150½	
17-8-28	2,160	1·6	34 5	..		
18-8-28	1,500	2 0	30·0			
20-8-28	1,200	2·0	24 0			
21-8-28	1,040	2 5	25 0			1
22-8-28	2,040	1 2	24 4		149½	1
23-8-28	1,590	2·5	39·7	0 280		2
24-8-28	1,680	1 6	26 8			2
25-8-28	900	2·0	18·0			2
26-8-28	.				151½	2
27-8-28	1,560	1 0	15 0			4
28-8-28	1,740	0 5	8·0	.		4
29-8-28	1,200	0·5	6 0			2
30-8-28	1,800	0·4	7 2	0·186		2
31-8-28	2,040	0·3	6·0			Omitted
					TOTAL	22

A perusal of Table I will show that this patient showed slight improvement as regards the quantity of urine passed, but decided improvement as regards the percentage of sugar in the urine and the total quantity of sugar excreted The blood-sugar was also reduced though it still remained above normal

Case 2—N G D, Hindu male, aged 50 years, a mild case of diabetes Patches of leucoderma all over the body Put on opium from 31st March, 1928 to 24th April, 1928, total quantity given 52 grains

TABLE II

Date	Urine			Blood-sugar per cent	Opium administered daily in grains
	Total quantity in c c	Percentage of sugar	Total sugar in grms		
31-3-28	1,500	5·0	75 0		1
2-4-28	1,080	5 5	59 4		1
3-4-28	840	6·2	52·0		1
4-4-28	1,200	5 0	60·0		2
5-4-28	1,080	4 5	48·0	0 186	2
9-4-28	1,680	2·0	33 6	..	2
10-4-28	1,080	2·0	21 6		3
11-4-28	1,080	1·6	17·2		3
12-4-28	1,470	0·2	29·4	0·169	4
13-4-28	1,200	2·0	24 0		4
14-4-28	1,200	1 2	14 4		4
16-4-28	780	1 0	7 8	0·113	4
17-4-28	1,500	0·6	9 5		4
18-4-28	1,260	0·6	7 5		4
19-4-28	900	Traces			4
20-4-28	1,200	Nil			4
21-4-28	960	Nil			4
23-4-28	1,140	Traces			1
24-4-28	720	1 8	12·9		
25-4-28	900	Nil	Nil		
26-4-28	900	Nil	Nil	0·110	Omitted
				TOTAL	52

It will be seen from the results given in Table II that the output of urinary sugar in this patient was reduced to nil under opium and the blood-sugar brought down to normal The general condition of the patient improved very much

Case 3—A M, Hindu male aged 49 years, a moderately severe case of diabetes Symptoms first appeared about a year ago, now suffers from polyuria, thirst, sciatica Put on opium from 24th January, 1928 to 9th February 1928, total quantity given 31 grains

TABLE III

Date	Urine			Blood-sugar per cent	Body-weight in lb	Opium administered daily in grains
	Total quantity in c c	Percentage of sugar	Total sugar in grms			
23-1-28	960	2·9	27 8		118½	
24-1-28	1,800	2·2	39·6			1
25-1-28	1,500	2·5	37 5		119½	1
26-1-28	1,440	2·5	34 7	0·176		1
28-1-28	1,440	2·3	33 1			1
29-1-28					121	1
30-1-28	1,380	2·3	31·7			2
31-1-28	1,200	2 0	24 0		117	2
1-2-28	1,260	1 6	20·1			3
2-2-28	1,380	1 8	24 8	0 170	116½	3
3-2-28	1,260	1·0	12·6			3
4-2-28	1,140	1 1	12 5			3
5-2-28						3
6-2-28	1,050	1 0	10·5			2
7-2-28	1,140	0·9	10·0			2
8-2-28	1,200	0 6	7 2			2
9-2-28	840	Traces				Omitted
					TOTAL	31

A perusal of the results given in Table III shows that there was a definite improvement regarding the output of the urinary sugar, though the blood-sugar did not show much decrease The general condition of the patient also improved

Case 4.—A K, Mahommedan male, aged 55 years, admitted to the hospital suffering from moderately severe diabetes. Sugar first discovered a short time ago, complains of weakness, pains in the body, frequency of micturition and thirst. Put on opium from 25th February, 1928 to 17th March, 1928 receiving a total quantity of 57 grains.

TABLE IV

Date	Urine			Blood-sugar per cent	Body-weight in lb	Opium administered daily in grains
	Total quantity in c c	Percentage of sugar	Total sugar in grms			
24-2-28	3,540	7·5	265·5	0·234	119¾	
25-2-28	4,080	7·1	288·6			2
27-2-28	3 480	8·2	288·0		116½	2
28-2-28	3,480	8·2	288·0			2
29-2-28	3 360	8·2	275·5		120	3
1-3-28	3,240	8·2	265·5	0·240		3
2-3-28	3 780	7·1	268·0			3
3-3-28	3 600	7·6	273·6			3
4-3-28	3 300	8·2	270·6		116	3
7-3-28	4 500	8·3	373·5			3
8-3-28	3 900	8·7	339·7	0·25	115	3
9-3-28	3 450	7·1	245·0			4
10-3-28	3 900	8·3	323·7			4
12-3-28	4,500	7·0	315·0			2
13-3-28	3 600	8·0	288·0			2
14-3-28	3 140	8·3	260·6			2
15-3-28	3,000	8·3	249·0	0·264		2
16-3-28	3 240	8·3	268·9			2
17-3-28	3 000	8·3	249·0			Omitted

A study of the results given in Table IV shows that there was neither any improvement in the output of the urinary sugar nor any reduction in the blood-sugar content. The general condition of the patient, however, improved slightly.

Case 5—S C M, Hindu male, aged 45 years, admitted to the hospital suffering from a moderately severe form of diabetes of long standing He was given treatment with opium from 5th June, 1928 to 25th June, 1928 receiving altogether 60 grains

TABLE V.

Date	Urine			Blood-sugar per cent	Opium administered daily in grains
	Total quantity in c c	Percentage of sugar	Total sugar in grms		
5-6-28	3,600	7 0	252 0		1
6-6-28	2,880	7·1	191 0		1
7-6-28	2,520	7·1	178·9	0 275	1
8-6-28	2,700	6 2	167·4		1
9-6-28	2,220	6·6	146 5		2
11-6-28	3,600	6·2	223 2		2
12-6-28	2,880	7 1	204 0		3
13-6-28	2,820	6 2	200·0		3
14-6-28	2,220	7 1	157 6	0·240	3
15-6-28	2,100	7·1	149·1		3
16-6-28	2,700	7 1	191 7		3
18-6-28	2,400	8·3	199·2		4
19-6-28	2,280	8·3	182·0		4
20-6-28	2,280	7 0	159 6		4
21-6-28	2,780	5 5	152·9	0·212	4
22-6-28	2,640	7 0	184 8		4
23-6-28	3,180	8 0	254 4		6
25-6-28	2,620	7·5	188·6		Omitted
				TOTAL	60

A perusal of Table V shows that there was no appreciable improvement either in the urinary output of sugar or any reduction of blood-sugar.

Case 6—R S, admitted to the hospital suffering from a severe form of diabetes of long standing Opium treatment was given from 2nd October, 1928 to 22nd October, 1928, a total of 83 grains being administered

TABLE VI

Date	Urine			Blood-sugar per cent	Opium administered daily in grains	Remarks
	Total quantity in c c	Percentage of sugar	Total sugar in grms			
2-10-28	1,980	5 0	99	0·390	1	Headache all day and drowsy
3-10-28	1,860	5 0	93		2	
4-10-28	1,800	5 0	90		2	
5-10-28	1,800	5·0	90		2	
6-10-28	1,740	5 0	87		3	
8-10-28	1,800	5 0	90		3	
9-10-28	1,980	5·0	90		4	
10-10-28	1,500	4 0	79		4	
11-10-28		5 0	75	0·400	4	Patient complains of a headache off and on, and drowsiness during the greater part of the day No other marked symptoms, tongue dry, intense thirst
12-10-28	1,440	5 0	72		5	
13-10-28	1,560	4 5	70		5	
15-10-28	1,500	4 5	60		5	
16-10-28	1,800	5 0	90		5	Patient very drowsy all evening Headache and intense thirst
17-10-28	2,100	4 0	84	0·440	6	
22-10-28					Omitted	
				TOTAL	83	

A perusal of Table VI will show that there was no appreciable reduction in the output of the urinary sugar The blood-sugar as a matter of fact increased with larger doses of the drug and the patient complained of more or less continuous headache throughout the treatment The general condition of the patient did not improve at all

Case 7—N K L, Hindu male, aged 28 years, suffering from a mild form of diabetes Polyuria started 3 months ago Complains of loss of strength, suffers from polydipsia, polyphagia and impotence Knee jerks increased Opium treatment from 12th May, 1928 to 6th June, 1928, received a total of 57 grains

TABLE VII

Date	Urine			Blood-sugar per cent	Body-weight in lb	Opium administered daily in grains
	Total quantity in c c	Percentage of sugar	Total sugar in grms			
12-5-28	2 820	3 1	37 1		129	1
14-5-28	2 280	3 5	79·8			1
15-5-28	2 580	3 8	98 0			2
16-5-28	1 680	4 5	75 6		128½	2
17-5-28	1 740	4 0	69 6	0 126		2
18-5-28	1 620	4 0	61 8			3
19-5-28	1 560	3·1	48·3			3
20-5-28					129½	3
21-5-28	900	4 0	36·0			3
22-5-28	1 560	1 4	21 8			3
23-5-28	1 500	2 0	30 0		129½	4
24-5-28	1 200	2·0	21 0	0·112		4
25-5-28	1 110	1 2	13 3			4
26-5-28	1 200	2 5	30 0			4
27-5-28					129½	4
28-5-28	1 020	0·9	9 1			2
29-5-28	1 200	2 5	30·0			2
30-5-28	1 140	2 0	22 8		129½	2
31-5-28	1 260	3·3	40 5	0 146		2
1-6-28	1 140	3 0	34 2			1
2-6-28	1 140	2 7	30 7			1
3-6-28					129½	1
4-6-28	1 200	3 0	36 0			1
5-6-28	780	3 0	23 4			1
6-6-28	840				129	Omitted

A perusal of Table VII shows that the output of the urinary sugar fluctuated a lot though it did come down to some extent The blood-sugar and the body-weight remained more or less stationary

Case 8—Mrs E, an Anglo-Indian female, aged 59 years, suffering from moderately severe form of diabetes Sugar first discovered two years ago Suffers from polyuria low fever in the evening and pain on the right side of the abdomen Opium treatment given from 13th September, 1928 to 5th October 1928, a total of 45 grains being administered

TABLE VIII

Date	Urine			Blood-sugar per cent	Opium administered daily in grains
	Total quantity in c c	Percentage of sugar	Total sugar in grms		
13-9-28	3 240	0·0	0·0	0 190	1
14-9-28	3 620	0 0	0·0		1
15-9-28	3 620	0 0	0 0		1
16-9-28					1
17-9-28	5 420	0 0	0 0		1
18-9-28	3 620	0·0	0·0		1
19-9-28	4 020	0 0	0 0		2
20-9-28	5 250	0 0	0 0	0 186	2
21-9-28	3 920	0·0	0·0		2
22-9-28	4 290	0·0	0 0		2
24-9-28	4,920	0 0			2
25-9-28	3 000	0·0			2
26-9-28	3 540	0 0	0·0		2
27-9-28	3 780	0·0	0 0	0 262	2
28-9-28	2 660	0·0	0 0		2
29-9-28	2 940	0·0	0 0		2
1-10-28	2 420	0·0	0·0		2
2-10-28	3 120	0·0	0·0		2
3-10-28	3 450	0·0	0 0		2
4-10-28	2 120	0·0	0·0		3
5-10-28	3,600	0·0	0 0		3
6-10-28	3 570	0·0	0·0		3

It will be seen from Table VIII that the urine was sugar-free and in this case the action of opium on the blood-sugar only was studied It will be

observed that the blood-sugar showed a definite increase The noteworthy feature in this case is that though the sugar in the blood rose to 0 262 per cent no sugar appeared in the urine showing a rise in the threshold level of the kidneys The general condition of the patient improved

Case 9—S P, Hindu male, aged 52 years, suffering from a mild form of diabetes of 10 years' duration Received treatment with opium from 9th March, 1929 to 4th April, 1929, a total of 107 grains having given

TABLE IX

Date	Urine			Blood-sugar per cent	Body-weight in lb	Opium administered daily in grains
	Total quantity in c c	Percentage of sugar	Total sugar in grms			
5-3-29	960	5 0	48·0		133	
7-3-29	1,260	5 0	63 0	0·240		
8-3-29	1,680	4 0	67·0			
9-3-29	2,100	3·3	69·0			2
11-3-29	1,680	3·5	58·0		134	2
12-3-29	1,920	2·0	38·0			3
13-3-29					135	
14-3-29	2,280	2·0	45 0			3
15-3-29	1,800	2·5	45 0	0 165		3
16-3-29	1,740	2 0	34 0			
17-3-29					135½	
18-3-29						4
19-3-29	2,340	1 0	23 0			4
20-3-29	2,160	0 5	10·0			4
21-3-29	1,680	1 0	16·0	0 176		4
22-3-29	1,740	1 0	17 0			4
23-3-29	1,380	1 0	14 0			4
24-3-29					135	4
25-3-29						
26-3-29	1,380	1 0	14 0			5
27-3-29	2,520	1 1	27 0		135½	5
28-3-29	1,080	2·0	21 0	0·173		5
29-3-29						6
30-3-29	1,620	1·0	16 0			6
31-3-29					132½	6
1-4-29	2,040	0·5	10 0			6
2-4-29	1,140	0·6	7 0			6
3-4-29	1,200	Traces			132½	6
4-4-29	1,680	Traces				Omitted

It will be seen that there is an appreciable reduction in the sugar output as well as in the blood-sugar The body-weight steadily increased in the earlier part of the treatment and the patient felt quite well All the subjective symptoms disappeared

Case 10 (*Case* 9 *on a second course*) —After opium was stopped for 3 days in Case 9 the sugar in urine again increased A second course of opium treatment was given to see if the administration of the drug will render the urine sugar-free again

TABLE X

Date	Urine			Blood-sugar per cent	Body-weight in lb	Opium administered daily in grains
	Total quantity in c c	Percentage of sugar	Total sugar in grms			
5-4-29	1,740	Traces				
6-4-29	2,340	Ft traces				
8-4-29	2,100	1·2	25 0		130	1
9-4-29	1,140	3·3	37 0			1
10-4-29	1,680	2 0	33 0		130¼	1
11-4-29	1,200	2 0	24 0	0·118		4
12-4-29	1,800	1 0	8·0			
13-4-29					132	
15-4-29	1,200	1 6	19·0			6
16-4-29	1,560	Nil	0·0			6
17-4-29	1,800	Nil	0·0		130½	6
18-4-29	2,280	Nil	0 0	0·130		4
19-4-29						
20-4-29	2,180	Nil	0·0			4
21-4-29					132	4
22-4-29	1,580	Nil	0·0			4
23-4-29				0·130		Omitted.

A perusal of Table X shows that after the administration of 25 grains the sugar disappeared from the urine again but a slight increase of blood-sugar occurred The general condition of the patient improved and the weight increased slightly

DISCUSSION OF RESULTS

Effect of opium on the urinary output—A study of the figures given in the above tables shows that so far as the daily urinary output is concerned the results were more or less variable Of the 10 cases under report in the present paper, there was appreciable reduction in the quantity of urine in one case only, slight reduction in 2 cases, variable reduction in 3 cases and practically no reduction in 4 cases The drug does not appear to exert any marked influence in reducing polyuria especially in severe type of the disease

Effect on the daily sugar excretion—In the early and mild cases of diabetes the drug exerted a well-marked influence in reducing the total sugar

output to a considerable extent and in some patients it entirely disappeared from the urine This was particularly the case with patients who suffered from a mild type of the disease (Cases 1 and 3) In moderately severe cases of diabetes of long standing, on the other hand the drug appears to have little or no influence in reducing the total output of sugar in the urine

Effect on the blood-sugar —Opium did not appear to produce much reduction in the amount of blood-sugar in early and mild cases of diabetes as it did on the urinary sugar In one case only (Case 2) where the blood-sugar was only slightly raised above the normal level, the blood-sugar was reduced to normal side by side with the disappearance of the urinary sugar but in two other cases (Cases 8 and 10) the blood-sugar was found to have definitely increased even though the urinary sugar was reduced to nothing or mere traces It would be reasonable to infer that the drug probably influences the renal threshold for excretion of glucose in some of the cases In the moderately severe cases of diabetes of long standing, the blood-sugar was either stationary or even increased after the administration of opium even though the output of the urinary sugar ran more or less at a constant level

Effect on the general condition of the patient —The general condition of patients taking the drug certainly improved in the majority of cases In almost every case there was a general feeling of well-being and cheerfulness and brightness which continued for some time even when the drug was suddenly stopped Constipation, however, was the only distressing symptom in almost every case and some sort of a saline purgative had to be given in those cases every day There was no appreciable effect of the drug on the patients' body-weight

Summary and conclusions

(1) Administration of small doses of opium—1 to 6 grains daily—produces a slight reduction in the total quantity of urine excreted in mild cases of diabetes In severe cases it does not reduce polyuria, thirst or frequency of micturition

(2) In the early and mild cases of diabetes opium has a well-marked effect in reducing the total daily output of sugar in the urine In some cases the sugar entirely disappears from the urine under its influence In severe cases of diabetes, however, it has no effect on the sugar output

(3) Opium has not much effect in reducing the amount of blood-sugar of diabetic patients, in fact in severe forms of the disease it actually increases it

(4) In some patients the administration of opium distinctly raises the renal threshold for excretion of sugar The sugar in urine disappears while that in the blood is increased It would not, therefore, be advisable to use opium in this disease

REFERENCE

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STUDIES IN THE PHYSICAL PROPERTIES OF DIFFERENT BLOOD SERA

Part IV

FILARIASIS

BY

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WITH a view to investigate the changes, if any, in the sera from the blood of filariasis patients from those of normal healthy persons, viscosity, density, surface tension and buffer action of sera from both the blood of filariasis patients and healthy persons were determined The result of these experimental observations shows that the sera from the blood of filariasis patients differ only slightly from those of normal persons with regard to some of their physical properties

SPECIFIC GRAVITY

The specific gravities of the clear sera were determined by adding a drop or two of the same to different mixtures of glycerine and water of known specific gravities The specific gravity of the mixture, in which the drop remained stationary, was taken to be the specific gravity of the serum During the time these observations were made, the room temperature varied from 27° to 28 5°C The specific gravities of twenty-four sera (*vide* Table I) from the blood of normal persons were taken The value of these varied from 1 025 to 1 027, while the specific gravity of sera from the blood of twenty-five

filariasis patients varied from 1 025 to 1 028 (*vide* Table II) It is concluded, therefore, that so far as specific gravity is concerned, there is no variation in the sera from the blood of filariasis patients from that obtained from the blood of normal persons

Viscosity

It has been pointed out in a previous paper (Chopra and Choudhury, 1929), that the applicability of ordinary methods of determining viscosity of true solutions to that of colloids, is not free from objection The difficulty consists in the theoretical interpretation of the effective resistance to the flow In our experiments, we compared the viscous resistance, as measured by the ordinary flow methods of normal and filariasis sera with a view to compare the rates of flow under similar conditions The same viscosimeter was used in all the measurements Experiments were done at the room temperature which varied during the course of these experiments, from 30° to 32°C The value of the viscosity of the normal sera varied from 1 40 to 1 58, while that of filariasis at the same temperature varied from 1 40 to 1 61 (*vide* Tables I and II) The normal sera were taken from the twenty-four healthy dog-bite cases and the filariasis sera were obtained from those under observation in Filariasis Research Experiments with normal sera were done at temperature of 27° to 28°C and the temperature co-efficient of viscosity of serum has been found to be approximately 1 5 per cent, even allowing for this correction, the viscosity of filariasis sera showed very little deviation from that of the normal blood sera

Surface tension

In a previous paper (Chopra and Choudhury, 1928) the surface tensions of normal and some pathological sera were determined The value of surface tension obtained for the normal serum at 31° to 33°C was 60 4 dynes per cm In that paper, surface tensions of sera from the blood of twenty filariasis patients were also reported The mean of these readings was 57 9 dynes per cm As a result of 35 more observations at temperatures between 30° and 32°C, it was found, the value varied from 55 4 to 58 2, though the minimum values obtained are very few in number (Tables I and II) It can be concluded from this that the surface tension of the sera from the blood of filariasis patients is slightly less than that obtained in case of the normal sera

Buffer action

The pH of serum and of mixtures of serum and hydrochloric acid were determined electrometrically at the room temperature by using an apparatus devised by McLendon (1916) Carbon dioxide was driven off by passing hydrogen slowly into the serum for about five minutes The reserve pH of the serum was found to vary in the case of normal persons from 7 9 to 8 2 whereas in the case of filariasis patients from 7 9 to 8 3 Results of experiments with

mixtures of hydrochloric acid and serum have been given in the appended tables It will be seen from these results, that the power of neutralizing acids is somewhat diminished in the serum from the blood of some filariasis patients (Tables III and IV)

TABLE I

Sera of Normal Indians

No	Specific gravity	Relative viscosity	Surface tension in dynes per cm
1	1 026	1 40	61 2
2	1 027	1 61	59·9
3	1 026	1 40	59·9
4	1·028	1 62	61 2
5	1 026	1 56	60·6
6	1 026	1 50	60·6
7	1 026	1 61	59·9
8	1 026	1 50	59·9
9	1 025	1 58	61 2
10	1 027	1 45	61 2
11	1 027	1 50	59·9
12	1 026	1 49	60·6
13	1 027	1 63	59·9
14	1 026	1 57	60·6
15	1 027	1 48	59·5
16	1 026	1 60	62·7
17	1 026	1 55	59 6
18	1 027	1 64	61 2
19	1 027	1 45	59·6
20	1 025	1 58	60·6
21	1 027	1 42	
22	1 026	1 55	
23	1 027	1 50	
Mean	1·0263	1 53	60·4

TABLE II

Sera of Pilariasis patients

No	Specific gravity	Relative viscosity	Surface tension in dynes per cm
1	1 028	1 10	57 4
2	1 028	1 10	58·2
3		1 52	57 8
4	1 028	1 51	58·2
5	1 026	1 53	55 1
6	1 027	1 45	57 1
7	1 026	1 15	57·8
8	1 026	1 50	58·1
9	1 025	1 45	58·1
10	1 025	1·50	57·3
11	1 024	1 55	58·8
12	1 027	1·58	58·1
13	1 026	1 50	58·8
14	1·027	1 42	58·1
15	1·027	1 58	58·8
16	1·027	1 48	58·8
17	1 026	1·55	58·1
18	1·027	1 50	57 3
19	1·027	1·50	58·1
20	1 026	1 55	57 4
21	1 026	1 45	58·1
22	1 026	1 48	57 3
23	1 026	1 50	57·8
24	1 026	1·55	58·1
25	1 026	1 50	58·2
26		1·50	56 4
27		1 58	55 4
28		1 50	57 4
29		1 48	58·1
30		1 45	57 4
Mean	1·0258	1·498	57 74

TABLE III

Buffer action of sera of normal Indians

No	Pure serum	pH 2 cc serum + 2 cc 01 NHCl	2 cc serum + 2 cc 0·1 NHCl
1	8·12	7·81	3·50
2	8·07	7·77	3·49
3	8·20	7·84	3·74
4	8·05	7·75	3·49
5	8·05	7·77	3·49
6	7·95	7·69	3·45
7	7·90	7·60	3·35
8	7·96	7·65	3·35
9	7·95	7·62	3·40
10	8·06	7·62	3·50
11	8·12	7·69	3·60
12	8·07	7·65	3·66
13	8·20	7·81	3·69
14	8·05	7·81	3·70
15	8·15	7·80	3·74
16	8·11	7·82	3·69
17	8·15	7·83	3·70
18	8·09	7·79	3·79
19	7·95	7·62	3·64
20	7·96	7·63	3·65
21	7·98	7·60	3·40
22	8·00	7·65	3·50
23	8·12	7·82	3·49
24	8·27	7·88	3·74
25	8·07	7·78	3·52
Mean	8·06	7·73	3·57

TABLE IV

Buffer action of sera of filariasis patients

No	Pure serum	2 c c serum + 2 c c 01 HCl	2 c c serum + 2 c c 01 NHCl
1	8·2	7·69	3 55
2	8·16	7 70	3·50
3	8·25	7 72	3·55
4	7·96	7 60	3 25
5	8·08	7·50	3·21
6	8·08	7 59	3 50
7	8·30	7·69	3·55
8	8·25	7·65	3 50
9	8·01	7 50	3·30
10	8·15	7 60	3·40
11	8·05	7 61	3 35
12	8·25	7·65	3·60
13	8·30	7 67	3 50
14	8·16	7·60	3 53
15	8·25	7·65	3 45
16	7 99	7 50	3 40
17	8·00	7 55	3·35
18	7·98	7 65	3·45
19	8·16	7 69	3 55
20	8·08	7 68	3·50
21	8·00	7 67	3 50
22	8·10	7·69	3·55
23	7 98	7 62	3 45
24	7·96	7 59	3·47
25	8·08	7 67	3·50
26	8·24	7·68	3 52
27	8·25	7 70	3·49
28	8 20	7 72	3·39
29	8·30	7·81	3·60
30	8·10	7 69	3 50
Mean	8·13	7 64	3 46

Discussion

A review of literature shows that very little work has been done on the different chemical constituents of filariasis serum It is, therefore, *a priori* not possible to discuss our results on physical properties Our results show that there is a change in some of the physical properties of sera from the blood of filariasis patients Consequently it is to be expected that there is also a variation in one or all of the different constituents of blood, namely proteins, cholesterol, lecithin, etc From the nature of the disease, these results also appear to be what might have been expected The chronic inflammation set up by the adult parasite is a localized defence reaction, but the chemical change in the circulatory lymph is more generalized Prominent among the chemical constituents of the chylous lymph is cholesterol Deposits of cholesterol are found in fresh lymph, hence there is a super-abundance of that lipoidal substance in the lymph (Stenhouse, 1925) It, therefore, appears that there should be an increase in cholesterol content of serum from the blood of filariasis patients It has been actually found by Boyd and Ray (1930) that the cholesterol content of the blood of filariasis patients is increased The lowering of surface tension may be accounted for by this variation in the cholesterol content, whether diminished buffer action is also due to high cholesterol content cannot be definitely said in absence of data on this particular point Had there been changes of a profound nature in other constituents of blood, it would have been detected by marked changes in viscosity and density also Since this is not the case we are of opinion that the blood constituents of filariasis patients, other than cholesterol, undergo very little variation from those of normal persons

Summary and conclusions

Density, viscosity, surface tension and buffer action of sera from the blood of normal persons and filariasis patients have been determined It has been found that the surface tension and buffer action of filariasis sera are somewhat diminished, while density and viscosity are not changed at all

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total alkaloidal yield It usually occurs to the extent of 5 to 6 per cent in Asia Minor opium, but in Indian and Persian opium it is present to the extent of 10 to 12 per cent It is present in opium in a free state though some authorities think it occurs in form of a meconate It can be readily separated from the other alkaloids

Preparation—When opium is extracted with water, morphine goes into solution, but the greater part of narcotine remains undissolved By exhausting the residue with dilute hydrochloric acid the alkaloid is removed as a hydrochloride, from the solution of this salt the base may be precipitated by sodium bicarbonate and crystallized from alcohol Narcotine may also be extracted from opium by boiling it with ether

Properties—Narcotine occurs as odourless, tasteless, shining prismatic crystals, having a melting point 174°-175°C The base is very slightly soluble in water, 1 in 25,000 at 15°C and 1 in 7,000 at 100°C It is soluble in alcohol, ether and in benzene, very soluble in chloroform, slightly soluble in amyl alcohol or light petroleum It also dissolves in lime or baryta water and slightly in aqueous ammonia It is lævorotatory—185° in alcohol and 199°9 in chloroform The solution in dilute acids is dextrorotatory Narcotine is a feeble monacidic tertiary base, its aqueous solution is neutral to litmus When heated with water in a closed tube at 100°C, or by prolonged boiling with water it becomes converted into opianic acid Nascent hydrogen converts narcotine into meconine and hydrocotarnine and on oxidation cotarnine and opianic acid are formed The synthesis of narcotine has been effected by treating an alcoholic solution of cotarnine and meconine with potassium carbonate or by simply boiling an alcoholic solution of these two substances

Pharmacological action

In most of our experiments rabbits and cats were used The animals were anæsthetized with chloralose administered by means of a stomach tube A dose of 0 1 gramme per kilo body-weight induced anæsthesia within an hour and a half on an average In experiments involving the respiratory system, urethane in dosage of 1 8 gramme per kilo body-weight was used intramuscularly This produces complete anæsthesia as a rule in 2 to 2½ hours, but occasionally it was necessary to supplement it with ether

Solutions used—Narcotine base, being very slightly soluble in water was difficult to get into a solution suitable for pharmacological experiments Unfortunately even soluble salts are difficult to prepare and are unstable In all our experiments, therefore, we dissolved the required quantity of the base in a few drops of dilute hydrochloric acid mixing it well with a glass rod, water was then added and a clear solution formed A bihydrochloride of the alkaloid is formed having a strongly acid reaction and in using this solution for pharmacological experiments we had to consider the possibility of the results being vitiated on account of the acidity In all experiments, therefore,

CIRCULATORY SYSTEM

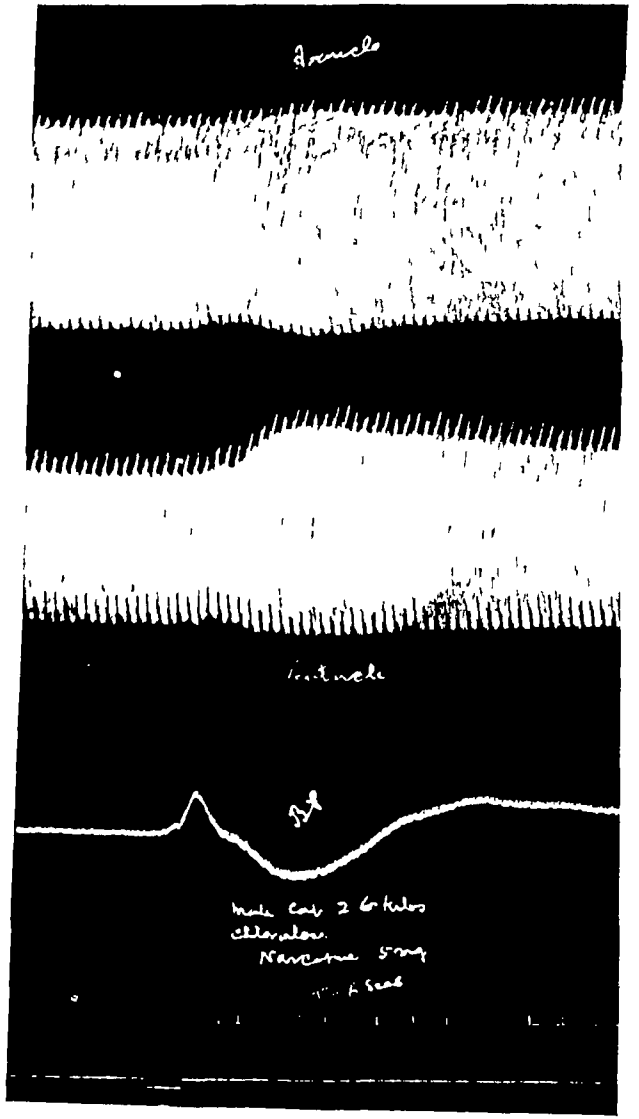
Effect on systemic blood-pressure—Plate I, fig *B* shows the effect produced by an injection of 5 mg of narcotine in the femoral vein of a cat Within 12 to 18 seconds of the injection the blood-pressure showed a sudden fall of a short duration After about a minute or so the blood-pressure again rose to slightly above the normal level and this level was maintained for about 2 or 3 minutes Injection of larger doses (10 to 15 mg) produced almost identical results excepting that the fall of blood-pressure was more marked Still bigger doses—20 mg or more—produced a spasm of the diaphragm and the blood-pressure showed marked oscillations Depression of blood-pressure is produced in decerebrated animals with the spinal cord destroyed showing that the cerebral and medullary centres do not play any part in this action Again it was produced even after the sympathetic nerve endings were paralysed with ergotoxine and the vagal endings were treated with atropine From these data we are justified in concluding that depression of the musculature of the vessel wall is responsible for the effect on the blood-pressure

Action on the pulmonary pressure—Plate I, fig *B* shows the effect of 5 mg of narcotine on the pulmonary pressure of a cat It will be seen that there is a small rise corresponding to the fall in systemic pressure This is due to a temporary influx of blood into the lesser circulation

Action on the blood vessels—We have already seen that an intravenous injection of narcotine produces a fall in the systemic blood-pressure This fall is still produced after the sympathetic ganglia are paralysed with nicotine and the sympathetic nerve endings are similarly treated with ergotoxine This at once suggests the possibility of the alkaloid having some direct action on the musculature of the vessel wall

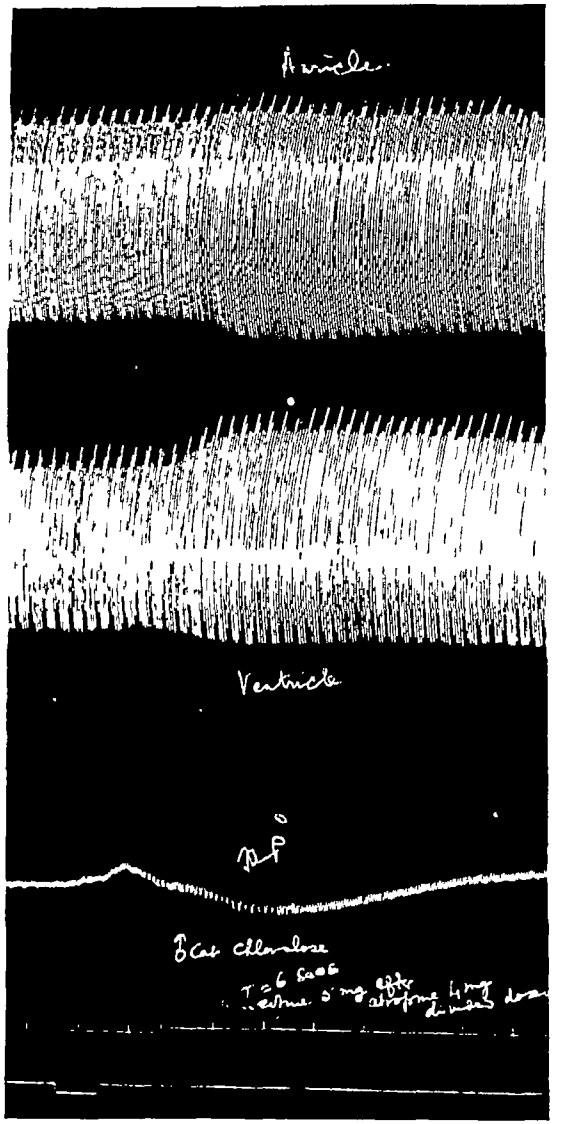
In order to determine any direct effect on the involuntary muscle of the blood vessels, a Trendelenberg preparation was put up After pithing a frog a canula was introduced into the aortic arch Through this the heart was perfused with a saline solution and the outflow was noted by counting the drops that emerged from the cut end of the inferior vena cava, sufficient time having been allowed for the preparation to give uniform results Narcotine was then added to the perfusate and the time taken for every 10 drops was recorded This showed a well-marked decrease showing that the alkaloid by its direct depressant action on the musculature had produced dilatation of the arterial system In warm-blooded animals such as the cat, addition of narcotine to the perfusate produced an appreciable dilatation of the blood vessels of the artificially perfused hind limb This dilatation was still present after the vasomotor nerve endings were paralysed with large doses of ergotoxine The drug, therefore, must have a direct action on the involuntary muscle fibres of the vessel wall

Plate II, fig *C* shows the changes occurring in the volume of the limb after 10 mg of narcotine were administered intravenously It will be seen that the limb volume is decreased when the blood-pressure falls but rapidly



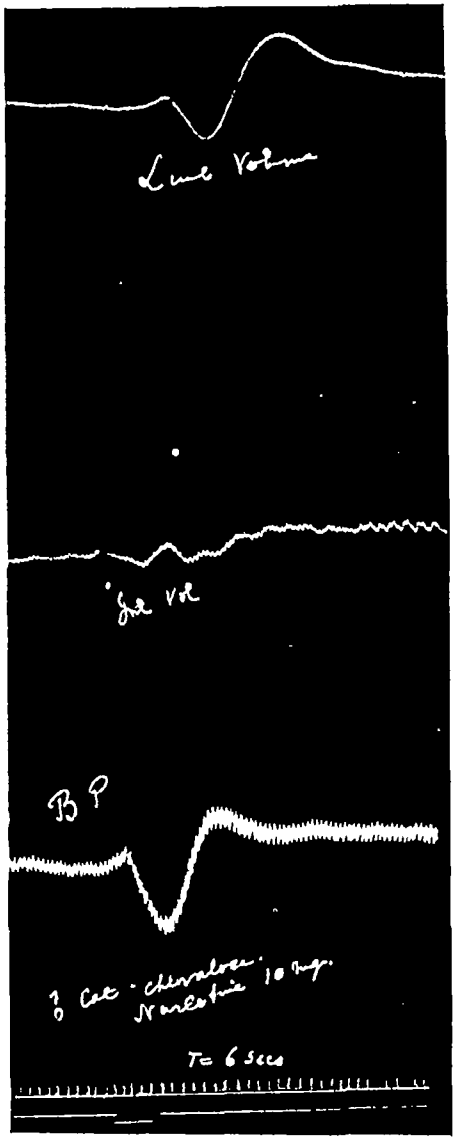
A

Fig *A* B P and myocardiograph tracings Note increase in the force and frequency of the beats of both the auricle and the ventricle Blood pressure shows a fall as



B

Fig *B* Myocardiograph tracings after the vagal endings in the heart are completely paralysed with atropine Note that the stimulation of the auricle and ventricle is still present showing that the vagus



C

Fig *C* B P intestinal volume and limb volume Note fall in B P, slight rise in intestinal volume and fall in limb volume followed by a rise

rises to well above the normal level, thus indicating a marked dilatation of the peripheral blood vessels

The effect on the blood vessels of the splanchnic area was studied by the effect produced on the volumes of such organs as the spleen, kidney and intestines Plate I, fig *D* shows changes occurring in the spleen It will be seen that there is a definite increase in the volume of the organs which lasts for some time In a few experiments a well-marked increase in the automatic movements of the spleen was also observed

Plate III, fig *A* shows the changes produced in the volume of the kidneys by an intravenous injection of 10 mg of narcotine The kidney volume in most cases showed a slight rise, in others it maintains its normal level In the majority of cases, however, the volume changes keep pace with the blood-pressure, i e, rises with the fall of blood-pressure and falls again when the pressure rises The intestinal volume also generally showed a well-marked rise (Plate II, fig *C*)

Action on the heart—Narcotine was given intra-hepatically in doses of 0 5 mg to pithed frogs, the movements of the heart being recorded on a moving drum There was a definite slowing of the heart and the force of the beat was somewhat decreased The action would appear to be directly on the heart muscle as paralysis of the inhibitory vagal mechanism did not in any way alter this effect

On the mammalian heart narcotine has a somewhat stimulant action Plate II, fig *A* shows the myocardiographic tracings of the effect produced by 5 mg of narcotine given intravenously in a cat It will be observed that there was a definite stimulation of both the auricles and the ventricles, the amplitude and force of the beat being increased, the rhythm remaining unaffected In order to determine whether this effect was due to an action on the nervous mechanism or on the myocardium, the connections with the central nervous system were several In some experiments, only the brain was destroyed by passing a stout metal seeker into the cranium, while in others both the brain and the spinal cord were destroyed In both these series there was no alteration in the effects produced by the alkaloid The stimulation of the auricles as well as the ventricles was still present showing that the effect was not central Again, when the vagi were divided in animals with brain and spinal cord intact, narcotine still produced its usual stimulating effect When the terminations of the vagi were paralysed by atropine, this effect also remained unaltered (Plate II, fig *B*) We can conclude, therefore, that depression of the inhibitory nervous mechanism is not responsible for the stimulant action of narcotine on the heart

Our attention was next directed to see if the accelerator mechanism played any part in the increased amplitude of the auricles and ventricles Injection of 5 mg of narcotine in an animal in which the sympathetic ganglia were previously paralysed with nicotine entirely abolished the stimulant action on both the chambers It would appear from this that the stimulant action is

probably located in the sympathetic ganglia of the heart Injection of 5 mg of narcotine after the sympathetic endings are paralysed with large doses of ergotoxine, instead of the usual stimulation, produces a slight depression of amplitude of both the auricles and the ventricle, the rhythm being appreciably slowed This is probably due to the action of the alkaloid on the musculature of the heart.

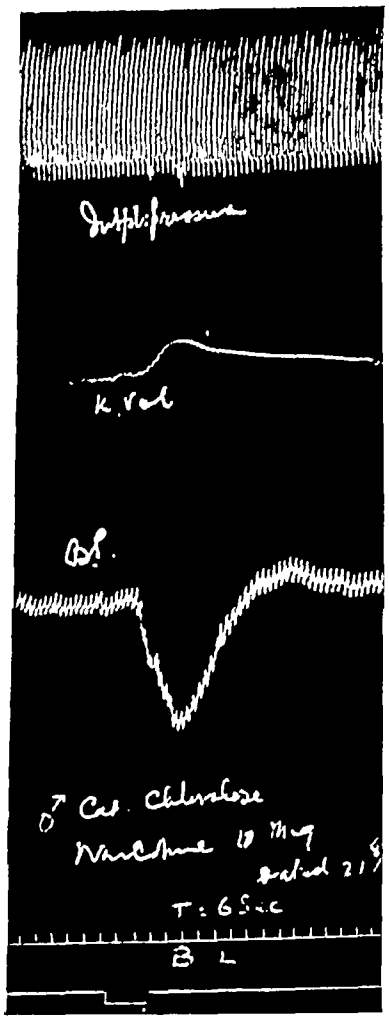
Plate III, fig *C* shows the effect of different dilutions of narcotine on the isolated cat's heart It will be noticed that a dilution of 1 in 100,000 depresses the amplitude very slightly This depressant effect becomes more marked and persistent as the concentration of the alkaloid increased This experiment supports the assertion already made that narcotine has got a direct depressant action on the myocardium

Action on the respiratory system

Some of the previous workers have referred to the stimulating action of narcotine on the respiratory centre Straub (1912) thought that the alkaloid was quite inactive by itself but merely potentiated the action of morphine These views were severely criticized by Meissner (1913) Macht (1915) showed by experiments on the CO_2 tension in alveoli of the lung that narcotine was not at all an inert alkaloid but had a definite stimulant action on the respiratory centre

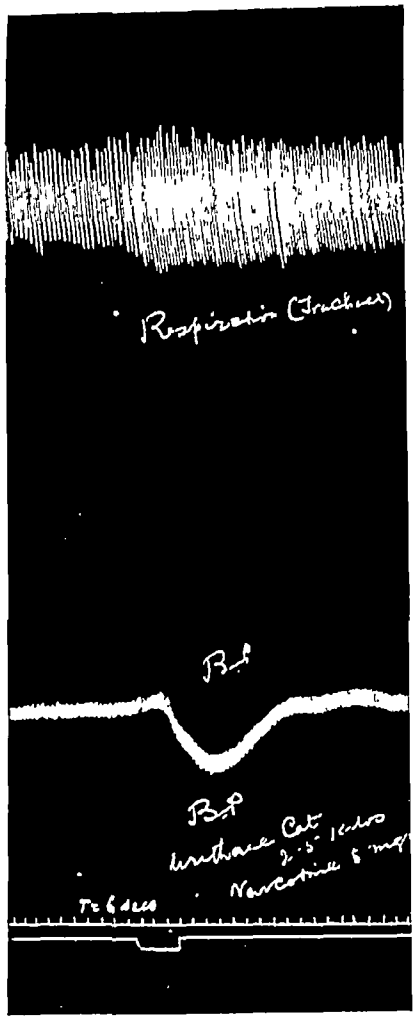
In order to study the direct action of narcotine on the respiratory centre, we recorded the tracheal pressure in cats One to two c c of a 1 per cent solution of the alkaloid was injected into the carotid artery so that it was carried directly to the brain and the medulla This produced not only a marked acceleration in the rate of the respiratory movement but both the expirations and inspirations became deeper and more powerful These facts indicate a direct action on the centre in the medulla

The effect on the respiratory centre was also studied by a modification of the method of Thomas and Frank (1928) The animal after being anæsthetized was put on artificial respiration, the diaphragm was completely separated from its costal border except the point of its posterior attachment which was kept intact All the blood vessels supplying this part were ligatured to render it completely avascular, care being taken to leave the branches of the phrenic nerves coming through the aortic opening in the diaphragm intact The diaphragm under these conditions, if it is constantly kept moist with warm saline, shows rhythmic contractions which are directly controlled by the centre in the medulla, and which can be recorded by attaching it to a lever Section of the phrenic nerves or freezing of the nerve stops the impulses coming from the centre at once and put an end to these rhythmic movements Injection of 10 mg of narcotine in such a preparation produced a marked stimulation of these movements showing that drug stimulated the respiratory centre (Plate I, fig *C*)



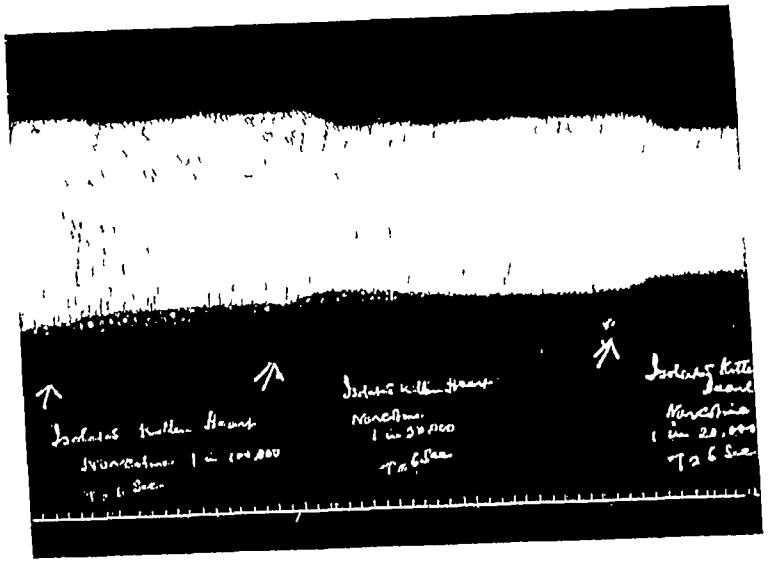
A

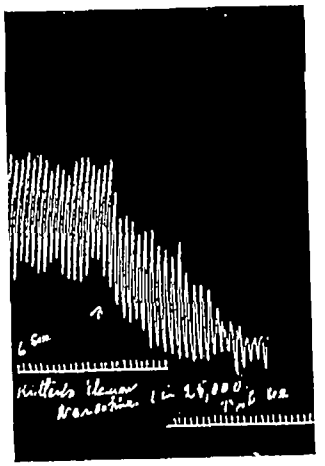
Fig. *A* Shows B. P. kidney volume and intrapleural pressure. Note the fall in B. P. and rise in kidney volume corresponding to the fall in B. P. Intrapleural pressure shows no change.

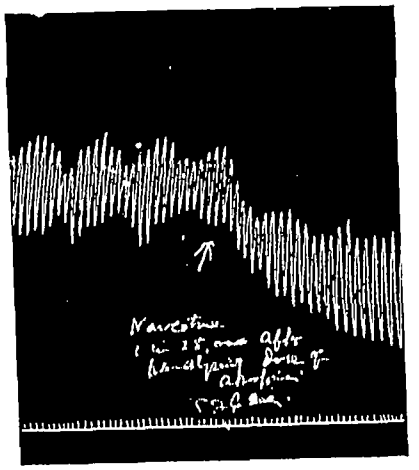


B

Fig. *B* Shows B. P. and tracheal respiration. Note fall in B. P. and stimulation of the respiratory movements.







D *E*

Fig. *C* Perfusion of isolated kitten's heart according to Langendorff's method —upstroke representing diastole and downstroke systole. Note depression of amplitude in varying concentrations of the alkaloid beginning from 1 in 100 000 to 1 in 20 000.

Figs. *D* & *E* Perfusion of isolated loops of intestine in a Dale's bath. Note marked inhibition of tone in Fig. *D*. The same effect is evident in Fig. *E*.

Plate III, fig *B* shows the tracheal pressure recorded by means of a Marey's tambour in a urethane cat It will be observed that an injection of narcotine produces an increase both in the frequency and in the amplitude of the respirations This stimulation is still observed after section of the vagi and after administration of sufficiently large doses of atropine to paralyse their terminations

The action of the drug on the bronchioles was tested by recording the intra-pleural pressure by means of a canula introduced through the ribs into the pleural cavity The animals were kept under artificial respiration from a mechanical pump in such a manner that the quantity of air pumped into the lungs remained quite constant Any constriction or dilatation of the bronchial muscles would be indicated by a decrease or increase in the amplitude of the respiratory excursions Narcotine given intravenously in 5 mg doses produced little or no effect (Plate III, fig *A*) in the majority of cases Intra-pleural pressure recorded in a decerebrated cat also gives similar results showing that the effect was on the musculature of the bronchioles and not on the nervous mechanism

Action on the central nervous system

There has been considerable difference of opinion regarding the narcotic properties of narcotine A perusal of the literature shows that a large amount of experimental work was done on lower animals by some of the early workers to determine the true action of the drug on the central nervous system Orfila (1853) dissolved the alkaloid in different solvents and administered it to dogs by the mouth He found that 0 5 to 1 3 grm per kilo in olive oil at first accelerated the respiration and then produced a condition of stupor which was followed by death Slight cramps of the extremities were noted Similar doses dissolved in acetic acid produced quickening of the respiration and spasms of the muscles all over the body which continued for hours Nodding movements of the head appeared in some cases and the head remained stiff and retracted At first the animal was quite conscious but stupor and coma supervened shortly before death When the same quantity of the alkaloid was dissolved in dilute hydrochloric acid or nitric acid the dogs did not show any toxic effects On the basis of these experiments this worker claimed that the nature of the action of narcotine depended largely on the solvent used Magendie on the other hand thought that the alkaloid was the excitant principle of opium According to him after administration of 0 05 gramme of narcotine per kilo in olive oil solution, the dogs showed definite convulsive movements instead of narcotic effects Chuvet (1857) claimed that the administration of 1 gramme of narcotine to rabbits was followed by slight tremors and increase in reflex excitability, after about 10 hours, the back and extremities became stiff and tremulous After 12 hours tetaniform convulsions occurred and the animal died

Von Schroeder's (1883) experimental study of narcotic action appears to be very instructive and thorough He found the action on the frog similar

to that of morphine There was definite onset of narcosis after a dose of narcotine but no sharp successive paralysis of any part of the brain was noticed as is produced by morphine In mammals the narcotic effects were inconsistent and less developed but tetanus-like contractions of the muscles were characteristic This tetanic state was considered by him to be due to abnormal reflex irritability of the spinal cord

It will thus be seen from the literature quoted above that most of the work done is not of very recent date and there is great diversity of opinion regarding the action of narcotine on the central nervous system Though evidence has been deduced in favour of its narcotic and hypnotic properties, the majority of workers seem to believe that the convulsant effect of the drug is much more pronounced than its sedative effect The wide variation in the experimental results and the diametrically opposite views put forward by different workers are most probably due to narcotine employed being impure and probably having a good deal of admixture of other opium alkaloids With a view to clear up the existing confusion we performed a series of experiments with chemically pure narcotine following mainly the line indicated by these early workers

Lower animals—We used mostly frogs and cats and our findings on these animals were fairly consistent and definite Frogs weighing on an average about 100 grammes were given the alkaloid by injection into the anterior lymph sac Doses of less than 0 1 grm produced no effect With increasing doses the animal showed within 10 to 15 minutes of injection slight rigidity of the anterior limbs, often starting on the right side, later the hind limbs were also affected, all the limbs lay stiff in an extended position With higher doses of 0 13 to 0 14 grm there was increased reflex irritability and tendency to opisthotonus or orthotonus These symptoms began to pass off in an hour or so but did not completely disappear With doses of 0 2 grm or more the frogs showed gradually increasing signs of irritability till after about 2 hours typical convulsions began to take place The limbs became stiff and rigid and the animal when put on its back could not resume its normal position The reflexes were markedly exaggerated and the animal was apparently completely paralysed and died in about 3 hours

In another series narcotine dissolved in dilute HCl was administered to cats in doses of 0 66 grm , 1 grm , 1 3 grms and 2 grms per kilo body-weight respectively by means of a stomach tube The first animal receiving the smallest dose showed no appreciable effect whatsoever The second animal vomited out a segment of a tapeworm after about 30 minutes and looked dazed and uncomfortable but showed no outward manifestation of excitement The third animal showed very definite symptoms of excitement It showed a tendency to rush wildly about, its intelligence and perception were dulled and later the muscles of the limb and back showed signs of stiffness The respiration was hurried from the beginning and there was a marked flow of thick, sticky and frothy saliva The fourth cat, which had 2 grammes per kilo, showed within

15 minutes marked restlessness and salivation The pupils were dilated and the respiration became very rapid In half an hour the head became retracted, twitchings of muscles occurred all over the body and all four limbs became stiff and in extended position Tetaniform convulsions started soon after, in fact the animal showed a typical picture of strychnic poisoning Cornea remained sensitive up to the last The animal died in about 2 hours' time due probably to spasm of diaphragm and accessory muscles of respiration, following convulsions Similar effects were also obtained in other animals

From these data we are inclined to believe that the stimulant and excitant effects of narcotine in animals are much more manifested than the hypnotic properties The parts of the central nervous system chiefly affected with smaller and larger doses are the medulla and the spinal cord, particularly the latter The hypnotic and sedative effects described by some of the early workers are in our opinion undoubtedly due to the alkaloid being impure

Action in man—The action of narcotine on human beings was investigated by the early workers either by administering the drug to volunteers or by taking it themselves Magendie believed it to be the excitant principle of opium, as after taking 0 3 grm he experienced some excitement and headache Baily could not get any definite effect with small doses Schroff (1856) noted in his patients that administration of 0 1 grm produced slight increase in pulse rate, followed by a decrease Temperature rose by 0 3°C and then came down again Soon after taking the alkaloid there was transient headache, humming sound in the head, flushing of the face, dilatation of pupil, increase in the frequency of respiration unpleasant sensations in the joints, sensation of heat in the chest, pleasant feeling, drowsiness and exhaustion After 2 hours the action disappeared Some of the later workers came to similar conclusions and believed that there was very slight, if any, hypnotic action, the pulse rate was slightly accelerated, the respiration increased in frequency and temperature rose slightly

The senior author has tried narcotine in a large number of patients suffering from malaria, diabetes, pneumonia, etc , in doses varying from 5 to 20 grains daily None of these patients showed any marked depression of the higher faculties as occurs in case of morphine, nor were there any signs of stimulation of the psychical areas of the brain The algesic areas, however, appeared to be somewhat depressed and sensibility of the patient to pain and discomfort produced by disease was decidedly diminished The patients looked more comfortable after the alkaloid was administered and said they felt better although the temperature was not appreciably affected There was no very marked stimulation of the respiration or the heart, no heightening of the reflexes, so that in therapeutic doses in man at any rate there were no outward signs of stimulation of the medulla or the spinal cord

One of the authors on two separate occasions took by the mouth 0 4 grm (6 grains) and 0 6 grm (10 grains) of the alkaloid He noticed a slight nauseating feeling which increased on moving the head There was a distinct

sensation of well-being for about an hour after the drug was taken No other action on the central nervous system was observed In another individual 8 grains were given after a hard day's work The sensation of fatigue greatly disappeared and this was followed by a feeling of lassitude and inclination to go to lie down if not to sleep No other effects were observed

From the experimental data given above, it would appear that narcotine when administered by itself has no marked action except on the algesic areas in the brain and in therapeutic doses has little or no action on the medulla Although narcotine has no direct effect on the cerebrum and higher centres of the brain, it has been shown to have synergistic action when given in combination with morphine Older investigators have shown that a dose of opium acts more strongly on the frog than the corresponding quantity of morphine contained in it Small doses of morphine in themselves inactive produce, when combined with small quantities of the subsidiary alkaloids, severe symptoms of poisoning (Gottlieb and V D Eeckhout, 1908)

Winternitz (1912) showed that hypnotic and sedative effects were produced in man by alkaloids of opium from which morphine had been completely eliminated The only alkaloid barring morphine that has a sedative effect in man is codeine which when given by itself has a feeble action In combination with the other alkaloids of opium, however, codeine produces as strong an effect as morphine The other alkaloids, therefore, appear to potentiate the action of codeine and of these narcotine has been shown to be the most important synergist Narcotine also has a well-marked synergistic action when combined with morphine so far as its action on the central nervous system is concerned Levy (1916) found that 3 mg of an equal mixture of morphine and narcotine exerted as great a narcotic action as 10 mg of morphine The greatest increase in activity is obtained when equal parts of narcotine and morphine are given together The decrease in perception of pain in man is also more marked when morphine and narcotine are combined The combination of one molecule of each with meconic acid has been recommended by Straub (1912) and named 'Narcophine' for use as a general analgesic Interesting experiments were conducted by Macht, Johnson and Bollinger (1916) and Macht, Herman and Levy (1918) to show that the increase in the pain-depressing action is due to the subsidiary alkaloids especially narcotine By measuring the strength of the induced current which would just produce a pain sensation from a single sensation point, they showed that 'Pantopon' and 'Narcophine' increase the threshold value of the effective stimulus more than the corresponding amount of morphine These observations have been confirmed and open a wide field for the use of narcotine This alkaloid occurs in large quantities in opium and up till now has been practically allowed to go waste in the preparation of morphine in India at any rate

We have already referred to the depressing effect of narcotine on the algesic areas in the brain, and from experience with this alkaloid we can fully corroborate the synergism which exists between narcotine, morphine and

narcotine and codeine Narcotine also possesses an antagonistic action to the depressing effect produced by morphine on the respiratory centre We will refer to it in more detail when dealing with the therapeutic uses of the alkaloid

Action on the genito-urinary system

The virgin uterus of a cat *in situ* showed a slight but a definite relaxation in its tone after an intravenous injection of 5 mg of narcotine but the automatic movements were not markedly affected The pregnant uterus does not show any marked changes either in tone of the muscle or its rhythmic contractions with such doses as 5 mg given intravenously The rhythmic contractions of the isolated virgin uterus of a cat did not show any appreciable changes in concentrations up to 1 in 50,000 The alkaloid in 5 to 10 mg doses given intravenously did not produce any effect on the urinary secretion, but the automatic movements of the bladder were slightly inhibited

Action on voluntary muscles—The effect of narcotine on the voluntary muscles was tested by studying the fatigue curves of nerve muscle preparations of the gastrocnemius of the frog Two muscle troughs were filled with normal saline and the nerve muscles were bathed in them Arrangements were made for simultaneous stimulation of the two nerves by induced shocks of equal strength, through the platinum electrodes To one of the troughs, narcotine was added in the strength of 1 in 50,000 while the other served as a control No difference was observed in the fatigue curves of the two muscles showing thereby that narcotine has got no appreciable effect on the voluntary muscles

Action on secretions—When taken by the mouth narcotine is tasteless, non-irritating and does not produce any reflex flow of the saliva In non-toxic doses of 0 5 to 1 0 grm, it does not stimulate the salivary secretion in cats After such large doses as 2 grms copious flow of thick viscid saliva was produced in about half an hour probably due to the action of the alkaloid on the salivary glands Other secretions such as those of the sweat, urine, etc, were not appreciably affected

Toxicity—The alkaloid does not possess remarkable degree of toxicity Its minimum lethal dose in the frog after injection into the anterior lymph sacs was found to be 2 grammes per kilo body-weight This is a bigger dose than that obtained by Issekatz and Barth which varied from 0 5 mg to 0 8 mg per gramme of frog Its minimum lethal dose in cats varies between 1 5 to 2 0 grms per kilo body-weight Large doses such as 2 to 3 grms can be tolerated in man without producing any other toxic symptoms than nausea, headache and vomiting

Discussion

From the aforesaid it would appear that the effect of narcotine on the central nervous system is generally not depressant as is the case with morphine

With the exception perhaps of the algesic areas, it produces a mild though a distinct stimulant action on certain parts of the cerebro-spinal axis The spinal cord undoubtedly shows stimulation which is most probably due to direct action of the drug on the nerve cells In toxic doses in cats the effect produced closely resembled those produced by toxic doses of strychnine The action on the circulatory system is again puzzling at first sight It has been shown that there is always a definite fall of blood-pressure after a dose of narcotine given intravenously This depression is very transient and is soon followed by a slight rise which is sustained for some time The fall in blood-pressure appears to be due partly to depression of the heart, as is supported by perfusion experiments, and partly to a lowered peripheral resistance brought about by the dilatation of the vessels of the splanchnic area produced by the direct depressant action of the alkaloid on the involuntary muscle fibres of the vessel wall The vasomotor nerves are not affected as the blood vessels dilate after sufficiently large doses of ergotoxine Apart from the action of the alkaloid on the musculature of the blood vessels involuntary muscle in other parts of the body is also depressed There is definite inhibition of the movements and relaxation of the plain muscles of the entire alimentary tract and this probably helps in splanchnic engorgement produced by this alkaloid

The subsequent rise of blood-pressure slightly above the normal level is probably brought about by a reflex stimulation of the vasomotor centre as a result of fall of the blood-pressure We could not prove direct action of the drug on the centre as in case of the respiratory centre The tendency for this subsequent rise to be sustained and the absence of rise after the vasomotor centre is destroyed both lend strong support to this view The stimulation of the auricles and ventricles seen in the myocardiograph experiments might again be attributed to the excitation of the vasomotor centre If we assume this to be the explanation we would expect the appearance of stimulation of the chambers later than the blood-pressure effect The stimulation, however, appears simultaneously with the depression of blood-pressure and this leads us to think that the accelerator nervous mechanism of the heart has probably some independent part to play The stimulation is entirely absent after the sympathetic ganglia are paralysed by nicotine and instead of the auricular and ventricular stimulation, there is definite depression after a paralysing dose of ergotoxine In the light of this experimental evidence, the possibility of sympathetic ganglion stimulation cannot be entirely ignored

Experiments show that narcotine has a distinct stimulant action on the respiratory centre in experimental animals Tracheal respiration in a urethane cat undoubtedly shows stimulation but this action does not appear to be very prolonged

The temporary inhibition of movements and relaxation of the tone of the plain muscles of the intestines and blood vessels are fairly constant findings The plain muscle of the uterus, however, does not show relaxation in all experiments, in the same dosage as in case of the intestines.

Narcotine in therapeutics

Narcotine was used in India in the treatment of malaria in the earlier parts of the 19th century but it was given up with the advent of the cinchona alkaloids, being recovered as a by-product in the preparation of morphine and it was hoped it could be utilized in medicine Its use against malaria was revived in 1895 on the suggestion of Sir William Roberts who in the report of the Royal Commission on Opium (1895) suggested that the narcotine in opium may have anti-malarial properties Quinine was not plentiful at that time and the medical authorities in India had to look for a cheap and easily procurable substitute Chopra and Knowles (1930) carefully studied the action of this alkaloid on a series of cases of different types of malaria and found that the drug had no effect whatever either on the malarial parasites or on the clinical symptoms of the disease

The analgesic properties of narcotine have not been sufficiently emphasized Although when given by itself it does not possess very marked sedative properties, when combined with morphine and codeine it potentiates their action to a remarkable degree, so that very much smaller quantity of these alkaloids are effective Further in cases of cough and other respiratory troubles such as asthma where morphine has to be used, narcotine in combination with morphine should be more valuable than the usual morphine and atropine combination As the alkaloid has a stimulant action on the spinal cord and the medullary centre its combination with morphine will, like atropine, counteract the depression of respiratory centre As a mild and harmless sedative narcotine would be a useful alkaloid in therapeutics We have tried it in pneumonia and other febrile conditions and found that it undoubtedly allays the discomfort and pain which commonly accompanies these conditions and its stimulant action on the respiratory centre is a distinct advantage The drug does not form a habit and that is a distinct advantage in its favour

Narcotine might have another field of utility in therapeutics We have already noticed that it has a depressant action on the involuntary muscle fibres all over the body It would, therefore, be very useful in relieving such spasmodic conditions as whooping cough, asthma, spasm of the intestinal musculature, bile duct, urethra, etc, and allaying cough, headache and pain of milder type It could also be advantageously combined with such purgatives as jalap, colocynth, etc, to prevent the griping and unpleasant pains of colicky nature which accompany their administration The inhibition of the peristaltic movement with this drug is not so marked as in case of morphine and it could be safely used in a number of conditions where morphine is contra-indicated Clinical trials are being conducted on these lines

Summary and conclusions

1 Narcotine is an important subsidiary alkaloid of opium inasmuch as it constitutes on an average 5 to 6 per cent of the total alkaloids It occurs

in large quantities as a bye-product in the manufacture of morphine and codeine and so far little or no use has been made of it in medicine

2 The alkaloid is readily absorbed from the site of injection, it does not produce much local irritation or necrosis of the tissues

3 Narcotine definitely inhibits the peristaltic movements of the gut It relaxes the tone of the involuntary muscle tissue all over the body, e g, uterus, bladder, gall bladder, etc, by its direct action on the muscle fibres

4 Given intravenously in animals narcotine produces a fall of systemic blood-pressure followed by a slight rise The fall is due to dilatation of the blood vessels, especially those of the splanchnic area, by its direct action on the musculature of the vessel wall The subsequent rise is probably due to reflex stimulation of the vasomotor centre to counteract the fall in systemic pressure The stimulation of the auricle and ventricle seen in myocardiograph experiments cannot be wholly explained by vasomotor stimulation and there is evidence to show that the sympathetic ganglion cells of the cardiac plexuses may be excited The depression of the heart seen in perfusion experiments is more than compensated by these two factors

5 Experimental evidence has been produced in support of the view that narcotine, unlike morphine, stimulates the respiratory centre in the medulla The plain muscles of the bronchioles is relaxed

6 The action on the central nervous system has been worked out by experiments on cold-blooded and warm-blooded animals The subjective sensations experienced by human beings after a dose of the alkaloid have also been recorded The conclusions drawn from these experiments are fairly definite and tend to show that the drug, in the animals at any rate, has a stronger action on the cord than on the brain The marked depressant effects of the narcotine on the central nervous system found by some of the early workers were probably due to the presence of other alkaloids of opium as impurities, due to imperfect technique

7 Narcotine has been shown to have a depressant action on the algesic areas in the brain and therefore lessens such symptoms as headache, pain in limbs, discomfort, etc, attendant on febrile conditions It undoubtedly potentiates the action of morphine and codeine so that much smaller quantities of these alkaloids would be effective if given in combination with narcotine

8 The voluntary muscles are not affected The secretions do not appear to be greatly influenced by narcotine in therapeutic doses In toxic doses there is a marked stimulation of salivary secretion but urine, sweat, etc, are hardly touched

9 The toxicity of the alkaloid has been worked out The minimum lethal dose is to be 2 mg per gramme body-weight in the frog and 1 5 to 2 0 grms per kilo body-weight in the cat Large doses such as 2 or 3 grms can be given in man without producing any marked toxic effects

10 The drug has no effect whatsoever on the malarial parasites or the clinical symptoms such as fever, rigors, etc, occurring in this disease

11 This alkaloid could be employed in medicine for its synergistic effects on the sedative properties of morphine, codeine, heroine, etc By itself it is beneficial in allaying cough, headache and other minor conditions producing pain of a milder type On account of its depressant action on the involuntary muscle tissue it would be useful in such conditions as asthma, whooping cough, spasm of muscular tubes such as the intestine, bile duct, urethra, etc Trials are being conducted on these lines

We are very grateful to the authorities of the Opium Factory at Ghazipore and particularly Mr Gaskel and Mr Rakshit for supplying us with sufficient quantities of narcotine for our experiments

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ON THE DISTRIBUTION OF LATHYRISM IN THE UNITED PROVINCES AND ON ITS CAUSE, WITH A DESCRIPTION OF A 4½ MONTHS FEEDING EXPERIMENT ON TONGA PONIES WITH BOTANICALLY PURE CULTURES OF *LATHYRUS SATIVUS* AND OF *VICIA SATIVA*

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1 DISTRIBUTION OF LATHYRISM IN THE UNITED PROVINCES

LATHYRISM (endemic spastic paralysis) is not a very rare disease in certain areas of the United Provinces During a recent period of 3 years, 22 cases of primary spastic paraplegia (i e, excluding all cases with a positive W R and those secondary to tubercle or growth) where lathyrism was suspected were admitted to the medical wards of King George's Hospital These cases resided as follows —Lucknow 6, Punjab 3, Bareilly 2, Pilibhit 2, Unao 2, Kheri 2, Bahraich, Basti, Sitapur, Hardoi and Rampur State 1 each Dr Hargovind Sahai with his many years' experience as physician in-charge of medical out-patients at King George's Hospital informed me that most cases he had seen came from the eastern districts of the province where the rainfall is high and the land low-lying, and that the disease was almost unknown in the western districts His experience was that Gorakhpur, Azamgarh, Balha, Lakhimpur and Sitapur provided most cases, whilst he had also seen an occasional case from Hardoi and Lucknow The Inspector-General of Civil Hospitals, United Provinces, was good enough to address 51 Civil Surgeons as to the frequency of lathyrism in the United Provinces Their replies indicated that there were two areas in the United Provinces (Balha and Allahabad) where lathyrism was not uncommon and one area along the Himalayan foot-hills (Gorakhpur, Gonda, Basti, Bahraich, Sitapur, Pilibhit) where the disease was recognized The

remaining districts of the province especially those in the western half were so far as was known practically free of the disease

As the frequency of the disease increased so definitely towards the eastern districts, culminating in Ballia, the most easterly situated of all, I asked the Inspector-General of Civil Hospitals of Bihar and Orissa for information as to its distribution in Bihar and Orissa The Civil Surgeons of this province were good enough to reply indicating that lathyrism was comparatively not uncommon in northern Bihar and practically did not exist south of the Ganges

The main Allahabad focus is around Shankergarh, where the people eat peas and rice This area is not far from Rewa State, where lathyrism is very common Obviously then lathyrism exists in the United Provinces to a greater extent than was formerly generally believed and I determined to have the home conditions of my next case more fully investigated

The opportunity soon presented itself when one Baldeo aged 40, was admitted into my ward from Kheri district with spastic paraplegia of 11 months' duration Baldeo stated that his father, uncle and only son had the same disease, whereas his wife and daughter were healthy (It is remarkable how frequently the women of the family seem to escape) His wheat crop had been damaged to the extent of 75 per cent—and his diet and that of his son had consequently consisted of gram 50 per cent and of lathyrus pea 50 per cent with a little barley At my request my house physician Dr R Chandra, MB, BS, Lucknow, was good enough to visit this area for a few days Dr Chandra furnished a most interesting report and found that 10 persons were affected with spastic paraplegia out of approximately 1,000 in this patient's village In the next village there were about 12 afflicted amongst 1,000 persons Similarly amongst other villages he visited the proportion with spastic paraplegia approximated 0 5 to 1 per cent The diet of these poorer labourers approximated lathyrus 50 per cent, barley 33 per cent, wheat 17 per cent Dr Chandra found several interesting examples of many cases of the disease in one family It was somewhat unexpected to find on investigation so definite a percentage of lathyrus cases in this area

2 Distribution of lathyrus cultivation in the U P

I applied to the Director of Agriculture for information as to the areas in the United Provinces under lathyrus cultivation The figures perhaps are not very accurate as to the actual areas sown by the villagers, and I am not inclined to attach much importance to them On the whole they indicated that more areas existed under lathyrus cultivation in the sub-Himalayan areas and in the eastern districts The actual figures in areas were Gonda 3,036, Badaun 1,454, Shahjahanpur 381, Moradabad 320, Bulandshahr 258, Farrukhabad 192, Etah 171, Bareilly 99, Bahraich 82, Hardoi 19, Bara Banki 18, Kheri 11, Sitapur 2

3 The disease amongst man and animals

Lathyrism inflicts cruel hardships on the afflicted It is usually the poor cultivator in debt, the bread-winner of a family, who with his intellect clear is smitten down in his prime with this incapacitating stiff paralysis of the legs The four stages, of cramps, of spastic paralysis, of bladder and rectal trouble, and finally of inability to progress except by crawling, follow each other in the worst cases with resentless sequence How apt are Shakespeare's words —

> 'Famine is in thy cheeks,
> Need and oppression starveth in thine eye,
> Contempt and beggary hang upon thy back,
> The world is not thy friend, nor the world's law'

Animals do not escape Fowls, pigeons and partridges eat the lathyrus pea freely and with apparent immunity Ducks are readily poisoned Of all animals the horse is especially susceptible Many outbreaks amongst horses eating lathyrus in feeding cakes have been reported and some such outbreaks have formed the subject of subsequent legal proceedings As long ago as 1820, the Paris Veterinary School warned farmers against using lathyrus peas for horses as it caused them to become roarers and to die if worked The main symptoms of equine lathyrism are (*i*) weak lumbar muscles ('gone in the loins') (*ii*) Roaring (from recurrent laryngeal palsy) (*iii*) Dyspnœa and sudden death on exertion (*iv*) Rapid and weak pulse (*v*) Debility, tremor and stiffness of the legs At rest the horse might appear quite well, but on exertion attacks of the above symptoms might appear and disappear equally suddenly The first signs in some horses is stumbling and staggering whilst at work so that, if pushed, they might stagger and even fall to the ground

4 The cause of lathyrism

Throughout the centuries in diverse countries from the time of Hippocrates, the disease has been attributed to the consumption of the lathyrus pea in sufficient quantity over a sufficient period of time What is the factor in a mixture of peas from a lathyrus crop that causes lathyrism? From experimental work Acton and Chopra indicated that the cause was a water soluble amine which was increased during germination and which could be removed from the lathyrus by soaking the grain for 24 hours in three changes of water (*Ind Med Gaz*, Nov 1922) But some crops of lathyrus will apparently produce lathyrism whilst other crops will not and the experimental work of many workers give discordant results as to the poisoning properties of lathyrus Anderson, Howard and Simonsen, a team combination of a medical research worker with a botanist and a chemist, by a well-planned research spread over many months, showed that a bazar specimen of lathyrus contained several distinct varieties of peas from which they separated amongst others two of special importance, *Lathyrus sativus* and *Vicia sativa*, which they cultivated in botanically pure culture They concluded that lathyrus was chemically

free from alkaloids and that controlled feeding experiments over long periods with ducks and monkeys showed that the grains are harmless and even nourishing to these animals They found that *Vicia sativa*, a weed commonly contaminating a lathyrus crop possessed alkaloidal bases One, divicine, produced a characteristic and fatal disease when inoculated into guinea-pigs *Vicia sativa* when fed to ducks caused death, and in monkeys produced characteristic nervous and muscular symptoms Though some of these symptoms have been described in human lathyrism they were not yet prepared to state that Akta is the cause of lathyrism in man (*Ind Jour Med Res*, XII, 4, 1925)

5 The Lucknow tonga pony experiments

Was the cause of lathyrism residual in *Lathyrus sativus*, or in the contaminating weed *Vicia sativa*? It is of the utmost importance to decide this point for in famine periods, when vast outbreaks of lathyrism are apt to occur, the effective preventative steps to be advised differ according to which pea is actually responsible Therefore I devised the following experiment and, in consultation with Major L A P Anderson, I M S, of the Central Research Institute, Kasauli, J T Edwards, Esq, of the Imperial Institute of Veterinary Research, Muktesar and Captain Hickey of the Civil Veterinary Department, United Provinces, put it into effect Three healthy tonga ponies were purchased in the bazaar and kept for a period in quarantine Their age (2 years 3 months), sex (male), height (10 feet 3 inches), girth (48 inches), shank (6 inches), weight (50 seers) and condition (fair) were approximately the same and the ponies were seen at fortnightly intervals by Captain Hickey who recorded their progress I saw the ponies daily at one feed and weekly at trotting and cantering exercise To each pony the same basic diet was given, namely, three pounds of barley with salt, a handful of lucerne when available and grass feeding *ad lib* To No 1 pony, a chestnut, named '*Vicia*,' three pounds of *Vicia sativa* daily were given mixed in the basic diet To No 2 pony, a roan named '*Lathyrus*,' three pounds of *Lathyrus sativus* daily were given mixed in the basic diet To No 3 pony, a stained chestnut named '*Control*,' the basic diet only was given The *lathyrus* and *vicia* were grown in botanically pure culture at the Institute of Plant Industry, Indore, Central India, and these pure strains I was able to obtain through the courtesy of the Director, A Howard, Esq, C I E A daily register was maintained of each pony's feeds, exercise and condition Each feed was mixed and given in the presence of Dr R S Lal, demonstrator in pathology at the King George's Medical College, to whom I am indebted for this considerable help The amount of any feed left by the animal was entered in the register It was expected from the experience of previous outbreaks of lathyrism in horses, that with this dosage of the pea symptoms of lathyrism would appear in one or at the most 2 months

The experiment started on 12th November, 1928, and was continued until 6th April, 1929, that is for 4 months 24 days, when it was forced to stop

owing to the exhaustion of botanically pure strains of the grain At the end of this period, all ponies were in equal and excellent condition and had put on weight At no time did they show any evidence of disease either at rest or during or after exercise

6 A PLEA FOR FURTHER RESEARCH

I conclude therefore that the experimental proof of the factor in *a lathyrus* crop which causes lathyrism is not yet complete and I urge that further research may be devoted to the solution of this important problem

THE CRYOSCOPY OF CALCUTTA MILK

BY

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THE cryoscopy of milk has been the subject of investigation for the last 30 years and the consensus of opinion is that the freezing point of milk is a more constant figure as compared with fat, solids-not-fat, etc It, therefore, forms a safe guide to the detection of even a small amount of watering In a recent paper A Van Raalte (1929) asserts 'the method of the freezing point of milk deserves international acceptance' In fact the Dutch Government has fixed in its Milk Decree the maximum for freezing point at not higher than —0 53°C, or in other words a milk whose freezing point is nearer to zero than —0 53°C is certainly adulterated E M Bailey (1922) of Connecticut, U S A, found for milk derived from individual normal cows a minimum depression of —0 53°C and a maximum of —0 566°C and similar figures for milk derived from normal herds In addition he found that disease or abnormal physical conditions in cows do not change the freezing point depression beyond the minimum limit of —0 53°C R L Andrew (1929) of New Zealand as a result of examination of 264 samples of milk derived from individual cows or herds has recently written that the freezing point ranges from —0 550°C to —0 560°C provided the determination be carried out according to his specified method This remarkable constancy of the figure prompted an examination of the method for Indian milks We have, however, found that his method although *suitable for* New Zealand is not at all applicable here where the laboratory temperature of 35°C during summer months precluded us from obtaining consistent results by this method

In view of all these facts, it is very surprising that the freezing point method has not been adopted as an official method in India where genuine milk is a rare commodity in a large city like Calcutta The only previous work in India is that of Dr Leather (1915) of Pusa in 1915 He made his determinations on Punjab and Pusa cows and buffaloes Altogether 77 samples were examined by him and most of these were derived from a single cow or buffalo His results are as follows —

	* Highest	Lowest	Average
Cow	0·577°C	0·518°C	0·542°C
Buffalo	0·562°C	0·521°C	

These figures cannot be accepted for Bengal without examination of local milks The largest milk concern at Calcutta is the Co-operative Milk Union which deal daily with over 100 maunds The supply is brought from villages situated within a radius of 30 miles from Calcutta Then milk is derived from the cows mostly of country breed which are kept under conditions differing from those of the Punjab and Pusa as regards climate and fodder In milk derived from individual cows we have found a wider range of variation in the freezing point than Dr Leather, viz, from —0 49°C to —0 59°C and in case of herd milk from —0 53°C to —0 59°C Eighty-nine samples of genuine milk of cow and buffalo obtained from various sources have been examined and in no case has there been any difference in the freezing point between the milks of the two animals The other constituents of milks have also been determined with a view to finding a relation between them and the freezing point A few samples of goat and human milk have also been analysed

Results —

Name of animal— Number examined—	Individual cow 50			Individual buffalo 20		
	* Max	Min	Avr	* Max	Min	Avr
F P	—0 590°C	—0·490°C	—0 519°C	—0 590°C	—0 510°C	—0·554°C
Fat (per cent)	9 0	3 0	5 4	8·7	3 9	6 7
S N F („ „)	9 54	7·92	8 85	12·47	9 21	10 08
Lactose („ „)	5 3	3 5	4·5	4·9	2 7	4 1
Chlorine	0·14	0 062	0·11			

* The term 'highest' or 'maximum' refers to the F P depression As a matter of fact its significance will be reversed if this is referred to the freezing point only, e g, —0·49°C is greater than —0·59°C

Name of animal—Number examined—	A big herd of cows 14			Mixed herd of cows and buffaloes 4		
	* Max	Min	Avr	* Max	Min	Avr
F P	—0 590°C	—0·530°C	—0·568°C	—0 590°C	—0·555°C	—0·575°C
Fat (per cent)	5 6	3·9	4 7	5 9	4 9	5 4
S N F („ „)	10 18	8·44	9·23	10·25	10·08	10·18
Lactose („ „)	4 80	3·40	4 40	5 1	3·9	4 6
Chlorine („ „)	0·094	0·060	0·08	0·078	0·061	0·064
Sp gr 15 5°C	1033 5	1029·5	1032 0	1034 5	1033 0	1033·5

Name of animal—Number examined—	Herd of buffaloes 1	Goat 4			Woman 4		
		* Max	Min	Avr	* Max	Min	Avr
F P	—0·590°C	—0·618°C	—0·565°C	—0 589°C	—0·595°C	—0·535°C	—0·566°C
Fat (per cent)	5·2	10·5	6 6	7 7	4 4	1 7	3 3
S N F („ „)	9 6	10·7	9·35	10·09	9·53	8 85	9·19
Lactose („ „)	4 9	4 9	4 1	4 4	6 6	5 5	6·0
Chlorine (, ,)	0·09	0·19	0 12	0·15	0·13	0·097	0 112
Sp gr 15 5°C	1033						

Our average F P of —0 549°C for individual cow's milk is very near what Dr Leather found for his Pusa and Punjab cows and our average for buffalo milk is slightly lower than his As regards goat's milk the average as well as the highest freezing point depressions obtained are higher than those of Josheph and Martin (1924) who found 0 575°C and 0 591°C respectively for Sudan goats This is the only other reference to the freezing point of goat's milk we have found Goat's milk is characterized by its slightly higher amount of chlorides as compared with milks of cow and buffalo whose chlorides rarely exceed 0 1 per cent No reference to the freezing point of human milk has been found It does not, however, differ much from the freezing point of cow

* The term 'highest' or 'maximum' refers to the F P depression As a matter of fact its significance will be reversed if this is referred to the freezing point only, e.g., —0·49°C is greater than —0·59°C

and buffalo milk and our other figures are comparable to those mentioned in the literature

All the samples were examined within 4 to 6 hours from the time of collection and their acidity ranges from 9° to 16 Hence no correction due to this on the freezing point was found necessary

The freezing point as a criterion of purity

The Bengal Food Adulteration Act lays down the minimum permissible percentage of fat as 3 5 in cow's milk and 6 in buffalo milk It also lays down that if the solid-not-fat falls below 8 5 per cent and 9 per cent in case of cow and buffalo milk respectively and the lactose below 4 4 per cent in both, the milk is presumed to be adulterated The following typical analyses of genuine milk done in the course of the present investigation may be examined in the light of the prescribed standards —

Individual cow milk	No I	No II	No III	No IV
Fat (per cent)	7 0	5 3	5 8	5 4
S N F („ „)	7 99	8·18	9 15	9·61
Lactose („ „)			3 70	5 30
F P	—0 577°C	—0·571°C	—0 573°C	—0 567°C

	Cow A		Cow B	
	Foremilk	Strippings	Foremilk	Strippings
Fat (per cent)	2 9	7 70	3 5	8·0
S N F („ „)	8 54	7 79	9 47	8·81
Lactose („ „)			5·30	5 30
F P	—0 578°C	—0 570°C	—0 587°C	—0 590°C

	Individual buffalo		Mixed cow	
	X	Y	P	Q
Fat (per cent)	7 0	5 5	1 2	5·6
S N F („ „)	9 8	9 9	8·44	9·20
Lactose („ „)	2 7	1 7	3 40	4 50
F P	—0·545°C	—0 545°C	—0 550°C	—0 560°C

In the case of individual cow's milk No I and No II, the S N F figure is much below the legal standard Their genuineness is, however, testified by their normal freezing point Compare the milk III with milk IV, milk X with milk Y, milk P with milk Q If we go by the lactose standard of 4 4 per cent, the milk III, X and P should be declared as adulterated But they are genuine milks as shown by their normal freezing point The case of foremilk and strippings affords a good illustration on behalf of the freezing point standard as the best test for purity In the former a very low fat content and in the latter low S N F would otherwise condemn the milk These instances go to show that the freezing point method alone can distinguish an abnormal, though genuine, milk from an adulterated milk It is to be noted that if the milk is drawn from the same cow in fractions (e g, foremilk and strippings), the freezing point remains practically constant This is very important as no other test except lactose can show this as illustrated in the milk from cow B

FIXING THE STANDARD FOR THE F P AND EXAMINATION OF SAMPLES FOR PURITY

Of the 50 samples of genuine individual cow milk examined, only 14 gave freezing point above —0 53°C and of these latter only 2 gave the maximum of —0 49°C In no sample of milk obtained from herds did it rise above —0 53°C even in pasteurized sample Pasteurization raises the F P slightly as will be shown

As the average F P of individual cow's milk is found to be —0 549°C it being the highest of all other averages, it is safe to suggest —0 53°C as the maximum F P in case of Calcutta milk, either of cow or buffalo or mixed milk

To find the possible amount of watering taking place, we examined samples from different sources Fifteen samples of milk from a 'milk federation' were examined of which only 6 were found to be absolutely water-free Some of these samples were purchased from depôts and some were sent to the laboratory by the federation The depot samples were on many occasions found to be slightly watered, but those sent from the federation office were found to be quite genuine A few surprise samples of theirs supplied to a Calcutta institution sometimes showed a distinct trace of watering The following are the typical examples in each case —

	Samples sent by the federation		*Depôt samples*	
			(1)	(2)
	30-8-29	19-9-29	13-8-29	14-8-29
Fat (per cent)	4 8	5 6	4 1	4 4
S N F („ „)	9·26	9·20	8·29	8·18
Lactose („ „)	4 60	4 50	3·90	4 20
F P	—0·54	—0·577	—0·49	—0·507

Added water calculated from the average freezing point of —0·55°C, in (1) 10·9 per cent and in (2) 7·8 per cent. Allowing for the added water the original milks would have the following composition —

	(1)	(2)
Fat (per cent)	4·6	4·8
S N F („)	9·30	8·87
Lactose („)	4·37	4·55

Institution samples

	8-8-29	16-8-29
Fat (per cent)	3·5	4·2
S N F („)		8·41
Lactose („)	3·4	4·0
F P	—0·357	—0·49
Percentage of watering	35·1	10·9

On the basis of the freezing point of the sample 16-8-29 evidently the sample of 8-8-29 has been further watered at the institution in addition to previous watering.

On allowing for added water, the original composition should be as follows —

	8-8-29	16-8-29
Fat (per cent)	5·4	4·7
S N F („)		9·47
Lactose („)	5·2	4·50

Twenty-seven samples of milk from a herd of cows or buffaloes or from a mixed herd were examined. These samples were from a local dairy. Only 13 were found to be genuine. In the beginning several morning samples were sent, all of which practically showed 7 per cent of added water. A few typical examples are given below —

Date	Nature of milk	Fat (per cent)	S N F (per cent)	Sp. gr. at 15·5°C	Lactose (per cent)	F P
9-9-29	Mixed cow	3·3	8·49	1029·0	4·1	—0·510
„	Mixed buffalo	7·9	8·59	1026·0	3·8	—0·515
12-9-29	Mixed cow	4·1	8·48	1028·5	3·9	—0·510
„	Mixed buffalo	5·2	8·47	1028·0	3·9	—0·510

On the complaint of watering being made, evening samples were sent to the laboratory most of which unfortunately turned rancid and consequently the freezing points went down

A few typical examples are as follows —

Date	Nature of milk	Fat (per cent)	S N F (per cent)	Sp gr	Lactose (per cent)	F P
16-9-29	Mixed buffalo	7 1	10·51	1034 0	3·4	—0 65
20-9-29	Mixed cow	5 0	9 67	1034 0	3 6	—0·615
,,	Mixed cow and buffalo	6·7	10 02	1033 0	2·8	—0 645

Further complaint being made of rancidity, morning samples were again sent, but this time the milking was done before a responsible officer All the samples henceforward gave freezing points typical of genuine milk The following are typical examples —

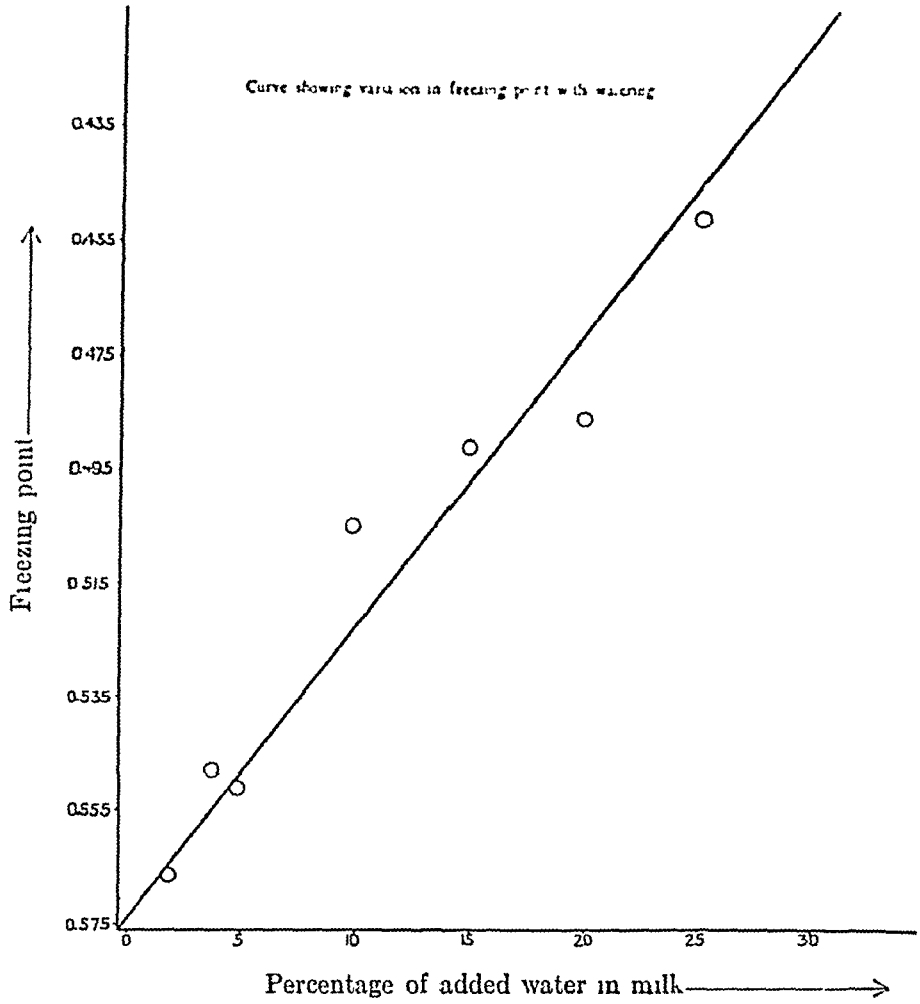
Date	Nature of milk	Fat (per cent)	S N F (per cent)	Sp gr	Lactose (per cent)	F P
23-9-29	Mixed cow	4 6	9 99	1033 0	4 7	—0 582
,,	Mixed cow and buffalo	5 9	10 08	1033 0	3·9	—0·555
24-9-29	Mixed buffalo	4 6	9·6	1033·5	4 7	—0 590
	Mixed cow	5·2	9·6	1033 0	4 9	—0·590

Relation between lactose and freezing point

In course of analyses of genuine milk we have noticed that as the lactose increases, the freezing point depression increases This is only true, however, between certain limits of lactose percentage, viz , 4 and 4 9 From 4 9 per cent upwards and also from 4 per cent downwards, the freezing point remains constant Between 4 per cent and 4 9 per cent lactose, the curve is a continuous straight line Graph 1 is drawn from the mean freezing points

obtained for varying quantities of lactose found in the genuine samples of mixed milk either of cow or buffalo or both

Graph 1



Examples —

Lactose (per cent)	F P
5 1	—0·590
4 9	—0·590
4 7	—0·581
4 5	—0·574
4 1	—0·555
3 8	—0 550
3 4	—0·550

In case of individual milks the relation between lactose and freezing point is not so marked In these it has been sometimes found that high freezing point is associated with low percentage of lactose This happens towards the end of the lactation period when the percentage of chlorides rises The following examples illustrate —

	(1)	(2)	(3)
Lactose (per cent)	4 1	4 2	4 4
Freezing point	—0·590	—0·590	—0 560
Chlorides as chlorine (per cent)	0·14	0·13	0 064

In Nos (1) and (2) low lactose associated with high chlorides gives quite a high freezing point whereas in No (3) high lactose with low chlorides gives normal freezing point In mixed milk of cow or buffalo the percentage of chlorides seldom exceeds 0 1 per cent and it generally varies between 0 06 and 0 09

The abnormal case of low lactose associated with high chlorides has also been noticed by other workers in case of the cows being kept on a poor or starvation diet

Henderson and Meston suggest the cause 'the mammary glands of the cow when unable to get the correct proportion of food-stuffs adjust the osmotic pressure by deriving an extra amount of chlorides from the blood'

A method for rapid estimation of chlorides in milk

The ordinary method of estimation of chlorides in milk from the ash is tedious Slow ignition is necessary in order to avoid loss of chlorides Chlorides (Richmond, 1920) may also be estimated by titrating milk directly with standard silver nitrate solution using potassium chromate as indicator In this method it is difficult accurately to judge the end-point The following method has been found to be quite easy and the results therefrom agree with those obtained from ash

Ten grammes of milk taken in a measuring flask is curdled with 3 drops of glacial acetic acid and heated on a water bath for 15 minutes and afterwards kept in an ice-box for another 15 minutes The volume is made up to 100 c c It is filtered The clear filtrate is cautiously neutralized with solid sodium carbonate (free from chlorides) An aliquot part is then titrated with standard silver nitrate solution using potassium chromate as indicator

Effect of pasteurization on freezing point and other analytical values

Pasteurizing is now used in Calcutta on a large scale Fresh mixed cow milk was pasteurized in the laboratory by heating it to 145°F on a water bath

and keeping it at this temperature for 32 minutes and then cooling it to 40°F This is the common method of pasteurization

Results —

	Fresh milk	On pasteurization
Fat (per cent) .	5·2	5·2
S N F („ „) ..	9·51	9·71
Lactose („ „) .	1·10	1·10
Freezing point	—0·564°C	—0·549°C
Chlorides	0·062	0·059

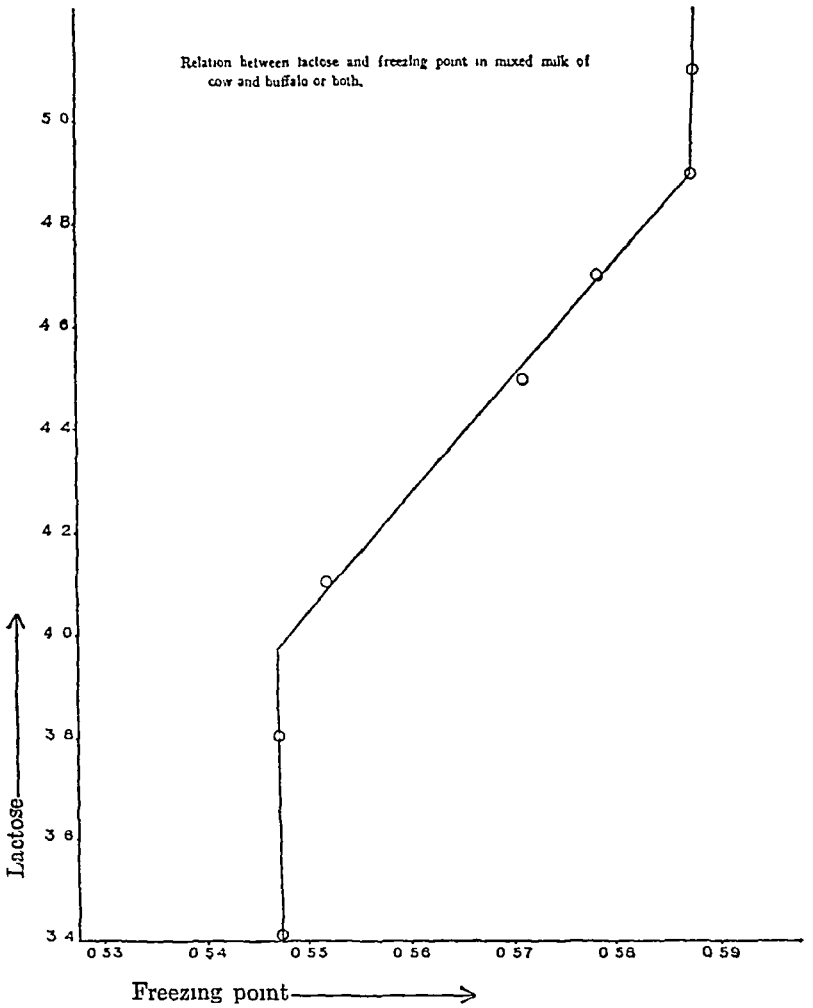
The S N F are increased owing to loss of water, whereas lactose diminishes owing to the destructive action of heat Consistent with this diminution of lactose the freezing point is raised by —0·015°C This is equivalent to 2·8 per cent of added water, —0·549°C being the average F P of individual cow's milk which is the lowest of all the averages, viz, that of mixed cow, mixed cow and buffalo and single buffalo A tolerance of 2·8 per cent of added water, if allowed, would raise the F P to —0·534 Under the circumstances, the maximum freezing point for milk fixed at —0·53 is not too high

Variation of freezing point due to watering

The freezing point of milk is raised due to watering The following data have been obtained experimentally by adding varying quantities of water to a genuine sample of mixed milk Its original freezing point was —0·575°C

Percentage of added water	Freezing point
2	—0·565°C
4	—0·543°C
5	—0·550°C
10	—0·506°C
15	—0·490°C
20	—0·485°C
25	—0·450°C

GRAPH 2



The trend of the curve in Graph 2 from the above is a straight line which is drawn so that it passes through the mean position of the various points The equation is $\frac{X}{Y} = K$ (constant) Hence K is called the coefficient of freezing point, Y is the difference of the freezing point from the original freezing point due to the percentage of added water X K as found here is 190

VARIATION OF FREEZING POINT DUE TO CHURNING

Monier Williams (1915) says that the freezing point of milk is not appreciably affected by the removal of fat In 'Dairy Chemistry' (1920) H D Richmond says 'Removal of fat has no practical effect on the freezing point' These statements were examined with reference to local milks Several samples of milk were churned in a Daisy churn until a fair amount of butter was obtained The butter was skimmed off as far as possible Both the separated milk and the original milk were next examined for fat, freezing point, etc

Typical results —

	No I milk		No II milk	
	Original	Separated	Original	Separated
Fat (per cent)	5·0	1·9	5·1	2·3
F P	—0·580	—0·530		
Lactose (per cent)	4·9	4·5	5·5	5·1

	No III milk		
	Original	1st churning	2nd churning
Fat (per cent)	4·5	2·7	1·5
F P	—0·546	—0·513	—0·480
Lactose (per cent)			

It appears that by taking out 60 to 70 per cent of original fat from the milk, a definite rise in the freezing point by —0·05°C to —0·06°C takes place This rise is associated with the diminution of lactose percentage by 0·5 This amount of lactose becomes adsorbed on the butter mass due to churning As the rise in the freezing point is higher than that corresponding to the diminution in lactose, it is evident that dissolved solid other than lactose is also adsorbed at the same time

Note on the method of reading the freezing point

The Beckmann's cryoscopic apparatus has been used throughout these determinations A precaution of which no mention has been made any where is found necessary to get concordant results We have found that although we tried to maintain the bath temperature very near the optimum temperature —4°C, with a slight change in this temperature both the readings for milk and water change simultaneously with time But the actual difference between the two readings which is the freezing point of milk remains constant throughout

Illustrative example —

		After 2 hours' interval
Milk reading	4·08	4·015
Water „	3·52	3·455
Difference (F P)	—0·56°C	—0·56°C

It is therefore advisable after each milk reading to have the corresponding water reading as quickly as possible To be still more accurate one should get the exact water reading at the very instant at which the milk reading is taken This can only be done by taking the mean of the two water readings, one before and one after the milk readings at an equal interval of time

We have checked this method against the cane sugar solution method as suggested by Monier Williams (1915) and found agreement between the two results

In taking the freezing points of milk or water the inner test tube of the apparatus with its contents is first rapidly cooled to about 1°C below the freezing point by placing it directly inside a stronger freezing mixture than the bath The contents are gently stirred as the temperature is going down The tube is then quickly lifted from this and the outside moisture quickly wiped off, it is then quickly inserted into the wider test tube immersed in the bath The stirring is continued gently until the mercury is seen to rise when it is momentarily stopped A supercooling of about 2°C in case of milk and 1 5°C in case of water is noticed and hence any necessity of correction due to this is thus obviated The thread becomes almost steady within half a minute and the stirring is renewed gently Further rise is noticed and the readings are then taken every half a minute The highest point to which the thread rises is the freezing point and here the mercury should remain steady for three minutes at least Often we find a gradual rise after this, but this is disregarded

Sometimes it may happen that the contents of the tube freeze before or immediately after it has been put inside the outer tube The readings thus obtained are not consistent and invariably higher than we got otherwise In such cases it is better to remelt the solid and start cooling again in the outside bath, but cooling should be stopped at a little higher temperature this time in order to avoid early freezing before the tube is put finally inside the outer tube

Andrew's method (loc cit) suffers from the above disadvantage in as much as the transference of the inner tube is recommended as soon as the mercury thread is seen to rise at the time it has been actually inside the stronger cooling bath By the time the tube is wiped and inserted in the outer bath, the very high laboratory temperature during summer months at Calcutta heats up the tube during transference and raises the freezing point quite appreciably Consistant readings are thus rendered impossible

Summary and conclusion

Freezing points of several samples of milk, e g, of cow, buffalo, goat and woman have been determined The maximum for cow and buffalo milk should be fixed at —0 53°C It is the best criterion of purity for abnormal milk possessing a low fat, S N F and lactose percentage Within certain limits

of lactose percentage, it varies directly as lactose in case of mixed milk There is a perceptible rise in freezing point due to churning and pasteurizing A modification in the usual method of determination has been suggested in order to get accurate results

Our thanks are to Mr D C De, B Sc , for having carried out several determinations of lactose, fat and specific gravity and also for having taken great pains in collecting genuine samples of milk We are indebted to Dr S Ghose, D Sc , for kindly lending his apparatus

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ON THE ELECTRICAL CONDUCTIVITY OF BENGAL WATERS

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THE electrical conductivity of ordinary water depends on the substances dissolved in it, and in most ordinary waters where the amount of saline substances dissolved is comparatively low, the electrical conductivity (E C) is directly proportional to the total amount of dissolved saline matter, commonly denoted as the 'total solids' The electrical conductivity can be determined in a few minutes, whereas the estimation of the 'total solids' takes a considerable time The ratio $\frac{\text{electrical conductivity (E C)}}{\text{total solids (T S)}}$ gives a factor which for any particular class of water is comparatively constant, and this factor having been determined for this class of water, the E C will enable the total solids to be quickly calculated Further any change in the amount of dissolved substances will be quickly reflected in the E C when such change might be difficult of detection by the ordinary methods of analysis An investigation into Bengal waters was undertaken with the object of determining the value of the E C and of the ratio F C/T S It has been found that this factor varies for different classes of water and for this purpose, Bengal waters may be divided into three categories —

(*a*) Flowing surface waters—such as rivers and springs
(*b*) Stagnant surface waters—tanks, bhils and shallow wells
(*c*) Subsoil waters—deep wells and tube wells

Table I gives the result of the examination of a series of river waters It is obvious from these that the readings of turbid waters are unreliable and that the 'total solids' figure must refer only to the 'dissolved solids' The E C of such turbid waters is extremely low, the suspended matter interfering

seriously with the conductivity Haziness due to slight quantities of suspended matter also affects the E C to a more or less considerable degree When the waters are clear, reliable results are got and the ratio E C / T S is here found to be between 11 and 12, the average being 12 5 (The E C is measured in megohms)

Table II gives the findings in a series of tanks and shallow wells There being comparatively free from suspended matters, the readings are proportional to the dissolved saline matter The ratio E C / T S varies from 11 to 14 and the average is 13 3 There are in this series some waters giving ratios lying outside the above range, e g Midnapore, Asansol and Alipur Duars These waters are not strictly alluvial and the explanation may lie in this fact On analysis these waters show a large amount of organic matter, the mineral matter (chlorides, carbonates and CO_2 free or combined) being comparatively small in amount

In Table III are collected the results of examination of a series of deep and tube well waters throughout the province and a few samples from outside The amount of dissolved saline matter in this is much higher than in Tables I and II, but the average figure of E C / T S is about 13 Exceptionally low ratios are given by a few places, e g , Jalpaiguri, Andaman Islands, where the low figures are probably due to a large amount of organic matter The effect of free CO_2 in excess in raising the ratio is shown in sample 36 As many ground waters are rich in free CO_2, this fact should be borne in mind in the examination of such waters

The electrical conductivity figure deserves a wider use than it possesses at present in routine water examinations Distilled water for use in laboratory work should not give an E C reading greater than 2, this gives a standard for distilled water difficult to fix in any other way

The Dionic Water Tester supplied by Evershed and Vingoles was used in these examinations and is a satisfactory instrument, the correction for temperature is carried out by adjustment of the columns of water under examination

TABLE I

No	Source of sample	Date	Total solids at 100°C	E C at 20°C	$\frac{E\ C}{T\ S}$	Remarks
1	River Hooghly at Chinsurah	12-3-29	96 6	340	3 6	Turbid
2	Hooghly River near Howrah Water Works	27-3-29	128 8	385	2·9	Do

TABLE I—*contd*

No	Source of sample	Date	Total solids at 100°C	E C at 20°C	E C / T S	REMARKS
3	River at Ramganj	11-5-28	607 0	113	0·19	Very turbid
4	River Burdwan	11-5-28	37·6	195	5 8	Turbid
5	River at Suri	18-5-28	288·2	104	0·48	Do
6	Raw River Water at Bankura	7-6-28	43 1	13	0·3	Do
7	River (Bankura) Raw Sump Well after Chlorination	9-8-28	85 5	165	1 9	Do
8	River Ramganj—Raw Service Reservoir	13-8-29	23·0	110	4 8	Do
9	River at Utterpara Raw	21-8-28	78·0	148	1·9	Do
10	Hooghly River at Serampore	28-8-28	104 0	142	1 36	Do
11	River Water at Suri	17-2-28	20·5	145	7 0	Hazy
12	River Bankura	5-3-29	50·3	365	7 27	Do
13	River at Suri	16-4-28	13·8	103	7 5	Do
14	Lower Ganges (Padma)	1-5-28	35 5	245	7 0	Do
15	River at Dacca	1-5-28	23·2	260	11·2	Slightly hazy
16	River Sitallaksha, Dacca	9-5-28	17 2	200	11 6	Do
17	Cossye River at Midnapore	9-5-28	13 0	80	6·1	Hazy
18	River at Suri after Settling with Coagulant	18-5-28	29·8	300	10·0	Very slightly hazy
19	Raw River Water at Ramganj after Settling with Alum	18-5-28	26 5	275	10 3	Do
20	Suri River Water after Settling	12-6-28	14 5	95	6 6	Hazy
21	Suri River Water after Chlorination	6-8-28	20 0 11 0	112 92	5 5 8·3	Do
22	Hooghly River at Serampore after Settling	28-8-28	13·5	142	10·5	Very slightly hazy
23	River Bankura after Settling	4-9-28	19 0	200	10·5	Hazy
24	River Water—Suri	8-12-28	18·0	165	9·1	Slightly hazy
25	River Hooghly at Chinsurah Water Works	10-1-29	39 0	400	10·3	Hazy
26	River at Burdwan Water Works	15-1-29	25 0	205	8·2	Very hazy
27	River at Berhampore Water Works	19-1-29	41 0	450	11 0	Slightly hazy

TABLE I—*contd*

No	Source of sample	Date	Total solids at 100° C	E C at 20° C	$\frac{\text{E C}}{\text{T S}}$	Remarks
28	Anglo-India Jute Mill River at Shamnagar, 24-Parganas	23-1-29	15 0	160	13·1	Very slightly hazy
29	River at Serampore	21-1-29	10 0	390	9 7	Slightly hazy
30	River at Bankura Water Works		31 0 Actual mineral found 23 17	310	11 0 11 5	Do
31	River at Mymensingh		13 0	80	6 1	Hazy
32	River at Chandpur		7·5	56	7 1	Do
33	River Water at Berhampore	15-2-28	27 0	100	11 8	Almost clear
34	Hooghly River at Kankinarah	21-2-28	28·6	380	13 3	Do
35	River Hooghly at Chinsurah after Settling Thoroughly	12-3-29	21 2	310	11 0	Clear
36	River at Burdwan	21-3-29	16 6	220	13 2	Almost clear
37	Hooghly River near Chinsurah	3-4-29	23 2	315	13·6	Do
38	River at Raniganj	10-4-29	16 6	205	12 4	Nearly clear
39	River at Burdwan	10-4-29	18·8	210	11 2	Do
40	Hooghly River at Berhampore	17-4-28	26 0	370	14 2	Clear
41	River (Bankura) Raw Sump Well after Chlorination	9-8-28	13 0	152	11 7	Almost clear
42	River Raniganj—Raw Service Reservoir	13-8-29	12 0	132	11 0	Do
43	River at Utterpara—Filtered	21-8-28	21 0	250	12 0	Do
44	Spring Barabakund, Chittagong	3-9-28	107 0	1,450	13·5	Clear
45	Pump Water Works, Bankura	3-9-28	9 0	132	14·7	
46	C W R—Berhampore Water Works	18-9-28	13 8	172	12 5	
47	River Water—Raniganj	10-12-28	16 0	200	12 5	Almost clear
48	River—Berhampore Water Works	11-12-28	28·0	410	14 6	Do
49	River at Krishnagar	11-12-28	24 0	290	12·1	Do
50	Spring at Cherrapunji	10-12-29	2 45	19·5	8 0	Do
51	Hot Spring—North Cachar Hills	22-12-28	42 43	475	11 2	Do

TABLE II

Shallow wells and tanks

No	Source of water	Date	Total solids at 100°C	E C at 20°C	E C / T S	REMARKS
1	Reserved Tank—Khulna	21-2-28	21 0	295	14 0	
2	Well—Maldah Sub-Jail	27-2-28	55 0	705	12·9	
3	Well—Pirgacha, Rangpur	27-2-28	18·2	240	13·2	
4	Tank—Dattapukur, 24-Parganas	28-2-28	22·0	285	13 0	
5	Tank—Natore Water Works	5-3-28	19·0	255	13 4	
6	Tank—Satkhira	12-3-28	19 5	280	14 3	
7	Tank—Sagor Police Station, 24-Parganas	16-3-28	63 0	860	13 6	
8	Well—Kurigram Sub-Jail	2-4-28	33 2	445	13·4	
9	Well—Midnapore Water Works	2-4-28	8·0	102	12·7	
10	Tank—Nator	2-4-28	16 8	252	15 0	
11	Well—Basirhat Police Station	10-4-28	88·0	1,220	13 8	
12	Tank—Krishnagar Police Station	17-4-28	23·0	300	13·0	
13	Tank—Khulna Water Works	17-4-28	18·0	270	15 0	
14	Tank—Dacca	1-5-28	36 0	430	11·9	
15	Well—Dacca	11-5-29	36 0	448	12·5	
16	Well—Maldah	2-7-28	12·5	1,400	11 2	
17	Khulna Tap Water	2-7-28	17·0	225	13·2	
18	Well at Chuadanga, Nadia	20-6-29	42 4	580	13 6	
19	Rajpur Well	20-6-29	52 2	605	11 6	
20	Tank—Khulna	13-8-28	19·5	235	12·05	
21	Well—Jessore	14-8-28	53 0	605	11 4	
22	Sump Well-Suri Water Works		10 5	120	11 4	Very slightly hazy
23	Well—Midnapore Central Jail	10-3-28	13·8	150	10·8	
24	Well—Dacca	1-5-28	42·0	400	9·5	Hazy
25	Well—Asansol	2-7-28	47·0	450	9·4	

A METHOD OF LOCATING UREASE WITHIN TISSUE BY A MICROCHEMICAL METHOD

BY

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[Received for publication, December 7, 1929]

To find out the exact location of urease within a tissue I have adopted the following method —When a solution of urea is added to any solution containing urease, then urea in the solution is split into ammonium carbonate It has been found out that this reaction takes place even if alcohol or acetone is present in the solution in a considerable proportion (80 to 85 per cent), though the rate of the reaction is then considerably slowed down In this concentration of acetone or alcohol the urease within cells is quite insoluble So when urea comes in contact with this urease, ammonium carbonate is formed exactly in those places where urease is present By using various neutral salt solutions for obtaining insoluble carbonates by double decomposition with ammonium carbonate, the exact location of urease may be found out These carbonates were rendered visible by various methods The salts of calcium, copper, lead, nickel and cobalt were used The outline of the method for each salt and its respective merits and demerits are discussed below

Calcium

Small pieces of jack bean seeds are soaked for two days in a solution containing 0 1 gm of calcium chloride, 0 1 gm of urea, 85 c c of ethyl alcohol and 15 c c of freshly boiled distilled water The brisk ebullition of carbon dioxide when the tissues are placed in acetic acid, shows the abundance of calcium carbonate formed by this reaction The tissues are then dehydrated and permanent sections are prepared by paraffin method After fixing the sections of the tissues on cover slide, the paraffin of the sections are dissolved away with zylol Then they are washed with absolute alcohol, lower grade of alcohol and finally with water Washing with distilled water is continued for some time, so that all calcium chloride or any other chloride or soluble salt present in the sections is washed away by the distilled water

They are then treated with 0·1 per cent silver nitrate solution in darkness for 5 to 6 minutes and then washed several times with distilled water to make them free from all soluble silver salts. The sections are now developed in light with formaldehyde solution or amidol and then washed with hypo solution and examined. As a control similar sections without urea are prepared and both are examined side by side. Sites of urease appear as black deposits.

The defects of the method are as follows —

(1) Silver nitrate forms insoluble salts with some acid radicles other than carbonate, such as chloride, bromide, etc.

(2) Nuclei of certain cells are stained by silver nitrate.

(3) It is very difficult to wash away silver from a section containing protein.

Other Metals

The use of silver nitrate may be dispensed with if a salt is chosen which is not injurious to the enzyme, and whose carbonate is coloured or may be rendered coloured by use of a reagent other than silver nitrate.

Nitrates of copper, lead, nickel, and cobalt were used for this purpose. Deposit of carbonate were obtained by using in the reagent the nitrate of one of these metals instead of calcium. Then permanent sections were prepared by paraffin method. After washing the sections several times with distilled water they were treated with a saturated solution of hydrogen sulphide for several minutes until complete blackening takes place.

Advantages and disadvantages of the methods are discussed below —

Copper.—Bluish green insoluble carbonate is formed. This when treated with saturated solution of hydrogen sulphide turns black. The method was discarded as copper has an affinity for proteins, so even when the sections are carefully washed they assume a diffuse brownish stain after treatment with hydrogen sulphide solution.

Lead.—The carbonate of lead which is formed in the tissue is of a very faint cream colour. After treatment with a saturated solution of hydrogen sulphide black lead sulphide is formed. By reason of the affinity of lead salts for proteins it has the same disadvantage as copper.

Nickel.—Insoluble carbonate of green colour is formed. When treated with a saturated solution of hydrogen sulphide the carbonate turns black. No perceptible precipitate of protein is formed when a solution of nickel nitrate is added to a protein solution.

Cobalt.—Insoluble bluish purple precipitate of cobalt carbonate is formed. When treated with a saturated solution of hydrogen sulphide the carbonate turns black. No precipitate is formed when cobalt nitrate is added to a protein solution.

Out of the above four salts cobalt nitrate was chosen as it has no action on protein. When treated with a dilute solution of sodium sulphide or

a saturated solution of hydrogen sulphide, a very insoluble sulphide of cobalt is formed The difference of colour between the nitrates and the carbonates of copper, lead, and nickel is very small but in the case of cobalt it is considerable When therefore a tissue containing urease is treated with urea and cobalt nitrate, the portion containing urease turns bluish purple and the remaining portion is coloured pale pink By this method the location of urease can also be made macroscopically

General technique of the method —

A small piece of tissue is taken and infiltrated with the following solution for about an hour —

Alcohol	80 c c
Cobalt nitrate	1 0 grm
Distilled water	20 c c

It is then transferred to a solution containing—

Alcohol	80 c c
Cobalt nitrate	0 5 grm
Urea	0 5 grm
Distilled water	20 c c

The tissue is left in the above solution for about 48 hours, but if the formation of precipitate is very scanty, it may be left in the solution for about 12 hours more If the formation of precipitate is very considerable it should be removed from the solution earlier The tissue is then prepared for the microtome by the new collodion method or by the paraffin method The sections are very carefully washed free from soluble salts with distilled water and then treated with a dilute solution of sodium sulphide or a saturated solution of hydrogen sulphide The deposits of cobalt carbonate in the sections will gradually turn black After the action is complete they are washed several times with distilled water and mounted permanently in the usual manner A control section for comparing the changes is prepared by leaving out urea from the cobalt nitrate alcohol solution The changes are as follows —

$$\text{Urea} + \text{Urease} \longrightarrow \text{Ammonium carbonate} \xrightarrow{\text{with cobalt nitrate}} \text{Cobalt carbonate}$$

$$\text{Cobalt carbonate} \xrightarrow{\text{with hydrogen sulphide}} \text{Cobalt sulphide}$$

By this method a fairly sharp picture of the location of urease within tissue may be obtained In the case of urease in the tissues of animal such as the stomach of the dog, better results may be obtained if 60 per cent alcohol is originally used in the cobalt-urea solution and then the percentage of alcohol in the reagent is gradually increased to 80 per cent Photomicrographs of some sections prepared in the above way are given in Plate IV accompanying this paper

Explanation of Plate IV

Fig 1 Section of a cotyledon of jack bean which was treated with alcohol-cobalt-urea solution The location of urease is seen as black reticular lumps engulfing small starch granules in their meshes

„ 2 Section of similarly treated radicle of jack bean

„ 3 Section of the stomach of a dog which was treated with alcohol-cobalt-urea solution Several black dots within the nuclei show the positions of urease

„ 4 Photomicrograph of a section of the stomach of a dog which was treated with alcohol-cobalt solution without urea and was used as a control section for comparing with section Fig 3

PLATE IV

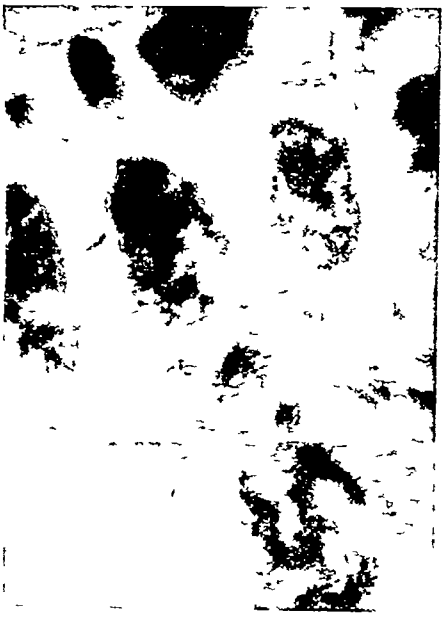

Fig 1

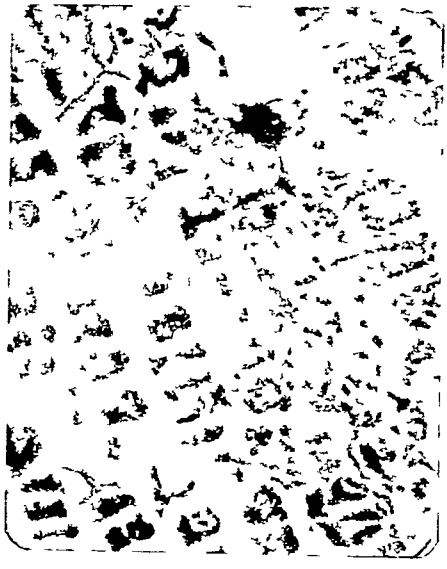

Fig 2

Fig 3

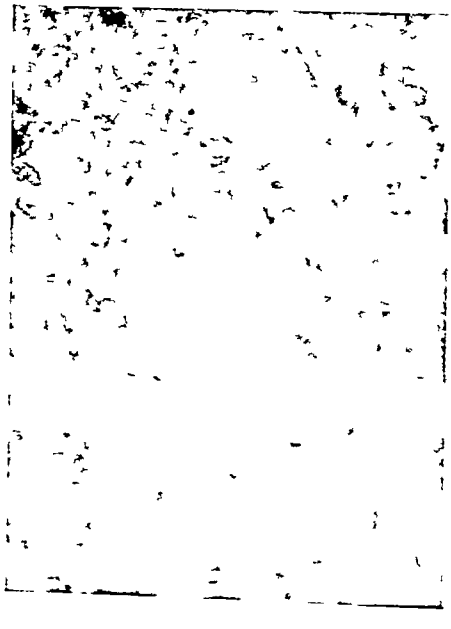

Fig 4

STUDIES IN DISINFECTION AND STERILIZATION

Part II

CHEMICAL CONSTITUTION OF TERPENES AND THEIR DISINFECTING PROPERTIES

BY

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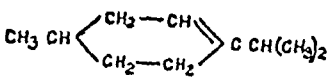
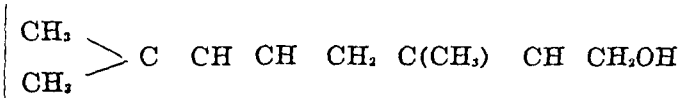
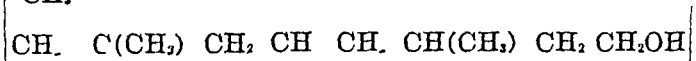
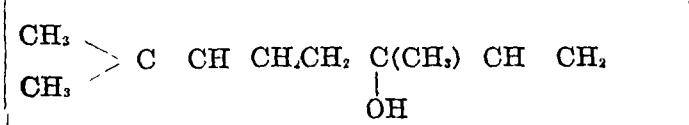
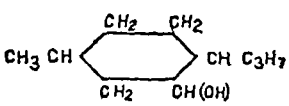
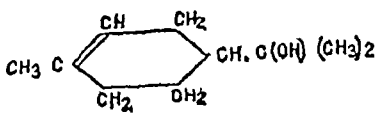
[Received for publication, December 14, 1929]

A REVIEW of the literature (1—15) shows that earlier studies on the relation of chemical constitution to disinfecting power were chiefly confined to simple compounds of the aliphatic and the aromatic series, coal-tar dye-stuffs and organometallic compounds Though the disinfecting properties of a great many terpenes and their derivatives are known, yet no attempt has, so far, been made to correlate them with the chemical constitution of the different compounds concerned An investigation was, therefore, started with a view to (*a*) evaluate the disinfecting powers of some representative terpenes and their derivatives under identical, controlled conditions, (b) study the nature of the relation of phenol coefficients obtained by the present author and some previous workers to the constitution of the compounds examined, and (*c*) suggest means for the enhancement of the disinfecting properties of such compounds as were found to be inactive either by introduction of suitable active groups into their molecules or by breaking them up into their active constituents as the case may be

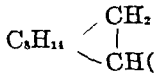
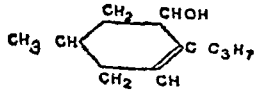
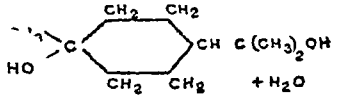
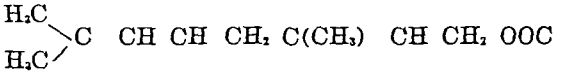
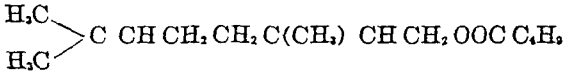
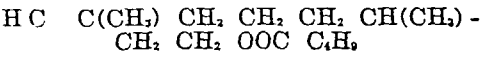
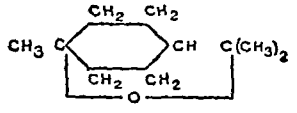
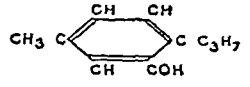
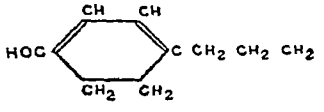
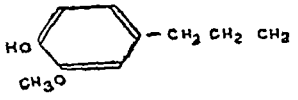
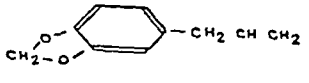
EXPERIMENTAL

The phenol coefficients of the various compounds were determined by the Rideal-Walker method using *Bacillus typhosus* (Hopkins) as the test organism Although the method is subject to much criticism, yet values obtained by it were found to be capable of indicating, with considerable accuracy, the relative germicidal activities of different substances of the same class when tested

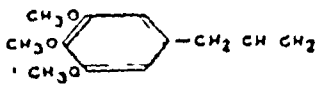
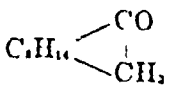
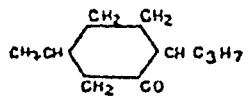
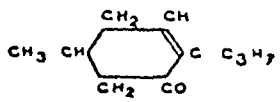
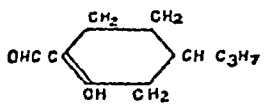
under rigidly controlled conditions. A mixture of pure sodium oleate and sodium carbonate was used as the emulsifying agent in preference to ordinary soaps as the impurities generally present in the latter would have vitiated the results to a considerable extent. Since in many cases the substances were solid, they were first dissolved in the minimum quantity of alcohol and then treated with the required volume of the emulsifying agent. In a few cases aqueous solutions of the substances were also used. Since it was observed that the author's figures were generally in close agreement with those of Penfold and Grant (1923—27) some of the latter's results for substances which were not locally available were incorporated in the following table with a view to illustrate the different points raised in the discussion —

Substance	Chemical formula	Phenol coefficient
Hydrocarbons —		
Camphene	$C_{10}H_{16}$	Less than 1·0
* Cymene	$CH_3\ C_6H_4\ C_3H_7$	8·0
Menthene		Less than 1·0
Pinene	$C_{10}H_{16}$	"
* Phellandrene	$C_{10}H_{16}$	"
* Limonene	$C_{10}H_{16}$	"
Alcohols —		
Geraniol		19·0
Citronellol		15.2
Linalol		14·0
Menthol		20·0
Terpineol		6·0

* From Penfold and Grant (*loc cit*)

Substance	Chemical formula	Phenol coefficient
Alcohols—concld		
Borneol		10 9
* Piperitol		13 0
Terpin hydrate		1 6
Esters—		
* Geranyl acetate		Under 1 0
* Geranyl valerianate		2 0
* Citronellyl valerianate		2 0
Oxide—		
Cineole		5 8
Phenols and their derivatives—		
Thymol		27 0
* Australol		22.5
Eugenol		12 7
Safrol		10 0

* From Penfold and Grant (*loc cit*)

Substance	Chemical formula	Phenol coefficient
Phenols and their derivatives—concld		
* Elemicin		Just under 1 0
Aldehydes and ketones—		
Camphor		7 0
* Menthone		10 0
* Piperitone		8 0
* Phellandral		9.25
Cumic aldehyde	OHC C_6H_4 C_3H_7	12 7
Citral	CH_3 \ / CH_3 C CH CH_2 CH_2—C(CH_3) CH CHO	17 9
Citronellal	CH_2 C(CH_3) CH_2 CH_2 CH_2—CH(CH_3) CH_2 CHO	13 0
Sesquiterpenes and their derivatives—		
Longifolene	$C_{15}H_{24}$	Less than 1 0
Santalene	$C_{15}H_{24}$	"
* Aromadendrene	.	"
B-Santalol	$C_{15}H_{23}OH$	1 4
* Eudesmol	$C_{15}H_{23}OH$	Less than 1 0
Trichloro derivative of a sesquiterpene		"
Nitro derivative of a sesquiterpene	..	"

* From Penfold and Grant (*loc cit*)

Discussion

It was observed that hydrocarbons were practically non-germicidal while their derivatives particularly those containing the groups,—CH,—CHO,—CO_2,—OR, and —O— had fairly high values It may, therefore, be inferred that introduction of such groups in the molecule enhanced germicidal power Thus, while the phenol coefficient of menthene was less than 1, the introduction of a —OH group (piperitol) raised the value to 13 and that of a ketonic group (piperitone) to 8 In a like manner, introduction of a—OH group into the molecule (thymol) of cymene raised its value from 8 to 27

A comparison of the phenol coefficients of alcohols with those of the corresponding ketones showed that the former were much more germicidal than the latter The low value of terpin hydrate suggested, however, that compounds containing two 'active' groups are feebly germicidal Terpin hydrate differs from terpineol in having one hydrogen atom and one hydroxyl group in place of the double bonds of the latter but while the coefficient of terpineol is 6 0, that of terpin hydrate is 1 6 This difference is probably due to the presence of an additional hydroxyl group in the molecule Similar observations were made (Morgan and Cooper, *loc cit*) with benzene derivatives Thus phenol, 1 0, resorcinal, 0 29, and catechol, 0 48

Unsaturation appeared to enhance germicidal power Thus, menthol, 20, thymol, 27, citronellal,* 13—, citral,* 17 9, citronellol,* 15 2, geraniol,* 19, phellandrial, 9, cumic aldehyde, 12 7

Hydrocarbons—All hydrocarbons with the exception of cymene gave very low values The observation should be interpreted to be the result of absence of active groups in the molecules

Phenols—Germicidal values of the different phenols and their derivatives decreased in the following order —

thymol		australol		eugenol		safrol		elemicin
(27)	>	(23)	>	(12 7)	>	(10)	>	(1)

It should be noted that in thymol the substituted alkyl groups (CH_3, C_3H_7) are heavier than those in australal (C_3H_5) and that one double bond of the benzene nucleus in australal is saturated Eugenol is more unsaturated than australal but contains two 'active' groups, —OH and —OCH_3 in the molecule Safrol which gave a lower value than australal also contains two active groups It thus appears that saturation of one or more double bonds of a compound lowered its germicidal efficiency to a less extent than the presence of a second 'active' group Presence of free phenolic groups appeared to increase germicidal activity as was evidenced by the higher value for eugenol as compared with that for safrol The very low value of elemicin suggested that the

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* It should be noted that citral and geraniol, in addition to possessing each one more double bond than citronellal and citronellol respectively, also differ from them with regard to position of common double bonds

presence of three 'active' groups as also the esterification of all hydroxyls combined to make it an almost non-germicidal body

Schaffer and Tilly (*loc. cit.*) observed that amongst the isomeric alcohols, the greatest germicidal activity is shown by that with the longest chain of carbon atoms, n-butyl alcohol thus being much more active than trimethyl carbinol. A study of their results shows, further, that even among alcohols having the same length of carbon chain, the germicidal activity depends on the position of the hydroxyl group, the greatest activity being shown by that having the longest chain counting from the hydroxyl group

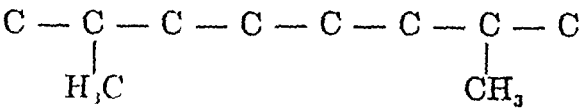
n-amyl alcohol- CH_3- CH_2 CH_2 CH_2 $CH_2(OH)$, 0·78,

methyl propyl carbinol—CH_3 CH_2 CH_2 $CH(OH)$ CH_3, 0·38,

diethyl carbinol—CH_3 CH_2 $CH(OH)$ CH_2 CH_3, 0·36

This may also explain why primary alcohols are generally more germicidal than secondary ones

The same is found to be the case with open chain alcohols of the terpene group. Geraniol, citronellol and linalol contain the same skeleton carbon chain —



Geraniol and citronellol have both the same length of carbon chain counting from the hydroxyl group but the former being more unsaturated gives a higher phenol coefficient. Linalol has a shorter carbon chain than the other two, but as it is more unsaturated than citronellol, its germicidal value approaches very nearly that of citronellol. The depression due to a shorter length of carbon chain is evidently neutralized by the presence of an additional double bond.

Hydro-aromatic alcohols

The fact that menthol has a higher value than terpineol even though the latter contains a double bond suggests that compounds having the hydroxyl group attached to the nuclear carbon atom are more reactive than those in which the hydroxyl group is in the side chain. The low value of borneol as compared with that of menthol suggests that bridged compounds are less active than those with straight chains.

The inactivity of terpin hydrate, as has been stated before, is probably due to the presence of two hydroxyl groups the disappearance of which with the formation of the oxide, cineole, causes it to show enhanced germicidal value.

Piperitol appears to be an exception to the general rule since it gave a lower value than menthol though it is less saturated than the latter.

Esters

Esterification appears to have reduced disinfecting power to a remarkable extent. This is particularly so in the case of geraniol, the phenol coefficient of

which dropped from 19 0 to less than 1 0 on its being converted to the corresponding acetate The results show that the reactivity of terpene alcohols is due to their free hydroxyl groups and suggest that where the starting material is an ester it will be possible to increase the disinfecting property by hydrolysis

Aldehydes and ketones

Aldehydes and ketones behaved in the same way as the corresponding alcohols Citral and cumic aldehyde are more unsaturated than citronellal and phellandral respectively and they gave correspondingly higher phenol coefficients Piperitone appears to be an exception, for it is less active than the corresponding saturated compound, menthone Camphor has the lowest value of the three ring chained ketones studied and in this respect it resembles borneol

Sesquiterpenes—Sesquiterpenes were all found to be inactive Neither the related alcohols nor derivatives containing active groups were found active This was probably due to their possessing much higher molecular weights than the ordinary terpenes Many instances are known in which germicidal power decreased considerably on increasing molecular weight beyond a certain limit The work of Morgenroth and his collaborators (1926) on quinine, of Leonard (1924) on alkyl resorcinol and of Penfold and Grant (*loc cit*) on higher aliphatic alcohols can be cited in this connection

Increase of molecular weight above a certain limit has evidently a depressing effect on the germicidal efficiency Sesquiterpenes molecules are heavier than those of true terpenes and it is possible that the inactivity of the former is due to this occurrence

Improvement of disinfecting power of terpenes

From the foregoing observations it may be inferred that the phenol coefficient of a terpene may be increased by (*a*) introduction of a single active group, particularly the hydroxyl, into the molecule, (*b*) reduction of active groups, if several, into one by esterification or otherwise, (*c*) making the compound more unsaturated, (*d*) lengthening the side chain, particularly that from the active group, (*e*) hydrolysis of esters, and (*f*) converting bridged compounds into straight chain ones

SUMMARY

(1) Terpene hydrocarbons were practically non-germicidal Compounds containing—OH,—CHO,—CO—and—O—groups were generally very active

(2) Unsaturation and increasing heaviness of side chain as reckoned from the position of active groups were observed to lead to enhanced disinfecting power while multiplication of active groups reduced the phenol coefficients considerably Compounds with active groups attached to nuclear carbon atoms were more reactive than those which have the same groups in the side chain

Esterification led to marked reduction in phenol coefficient Bridged compounds were less reactive than the corresponding ones with straight chains

(3) The inactivity of sesquiterpenes is probably due to their possessing high molecular weights as compared with ordinary terpenes Even the introduction of active groups did not improve their phenol coefficients to any appreciable extent

(4) Methods for improving the disinfecting powers of terpenes and terpene derivatives are suggested

The author's thanks are due to Dr R V Norris for his interest in the progress of the work and criticism

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STUDIES IN DISINFECTION AND STERILIZATION

Part III

COMPOSITION OF ESSENTIAL OILS AND THEIR DISINFECTING PROPERTIES

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IN the course of the previous investigations (De and Subrahmanyan, 1930) it was observed that (*a*) certain Indian essential oils possess valuable disinfecting properties and can advantageously replace coal-tar disinfectants in medicine and surgery, and (*b*) disinfecting powers of terpenes and their derivatives are determined largely by the chemical constitution of the compounds concerned Since terpenes are, normally, the main constituents of essential oils, it appeared possible, by a study of the composition of the latter, to obtain estimates of the disinfecting powers of the oils concerned Such a study, it was expected, would further help to determine (*a*) the relative effects of the major and the minor constituents of essential oils on the disinfecting powers of the latter, and (*b*) whether knowledge of changes in the composition of an oil, as determined by chemical analyses, will help to estimate the corresponding variations in the phenol co-efficient of a disinfectant prepared out of it and thereby enable the manufacturer to maintain the disinfecting power of his preparation at a constant value

Although the chemical constituents and the disinfecting properties of a great many essential oils and perfumes prepared out of them have been determined, yet study of the association between the two is exceedingly difficult to carry out (*a*) Such specimens of oils as were purified with care and were examined completely for their constituents were rarely ever used for determining their disinfecting powers (*b*) Such oils and perfumes as were generally used for determination of phenol coefficients were mostly of indeterminate composition (*c*) Where the oils were the same and their compositions at least

partly known, the methods adopted by different authors for evaluating their phenol coefficients differed so markedly for each other and the results obtained were so divergent that in many cases no comparative estimates could be obtained Thus, while Martindale (1910) and Bryant (1921) respectively obtained one set of values by the 'Lancet' method, Rideal, Rideal and Server (1928) and Reynolds (1925) respectively obtained entirely different values for the same oils by the Rideal-Walker method (*d*) Even where the method of determining the phenol coefficient was the same, individual variations in apparently minor details such as mode of preparation, age and dilution of emulsions of the different oils led to such marked differences that it becomes exceedingly difficult to compare the results obtained by any two independent authors without allowing for fairly big margins of error Thus, although De (*loc cit*) and Penfold and Grant (1923—27) adopted the same (Rideal-Walker) method and their other results were generally in close agreement, their figures for geraniol were 19 0 and 21 0 respectively and for citral, 17 9 and 19 5 respectively, involving about 5 per cent error on either side of the mean

In addition to the above, it is possible that even carefully distilled and purified specimens of oils from botanically identical plants, but grown in different countries may be entirely unrelated to each other in composition Thus, Pillay, Sanjiva Rao and Simonsen (1928) observed that the oil from the Indian 'botha' grass (*Cymbopogon Coloratus* Stapf) had the following percentage composition —*l*-camphene, 15, *l*-limonene, 7, camphor (?), trace, *l*-borneol, 8, geraniol, 10, sesquiterpenes, 35, sesquiterpene alcohols, 8, sesquiterpene oxide (?), 2 —3, and unidentified, 14 This composition was entirely different from that of the oil from the same grass grown in Fiji and which had the following percentage composition —*l*-limonene and other terpenes, 7, aldehydes (mainly citral), 40, and geraniol, 23 (Goulding and Earl, 1914) Such a difference in composition may be expected to affect, markedly, the properties of the two oils so that although they bear the same name, they will differ from each other in their disinfecting powers The Indian oil is composed mostly of hydrocarbons and sesquiterpene derivatives, which have practically no germicidal value it contains geraniol and borneol, which are active, only to the extent of 18 per cent The Fiji oil, on the other hand, contains, to the extent of 63 per cent aldehydes (mostly citral) and geraniol, which are highly reactive It may, therefore, be expected that the former will have a low germicidal value and the latter, a fairly high one

The foregoing illustration while showing that the name of an oil may not, by itself, convey a correct estimate of its germicidal value shows, on the other hand, that a knowledge of its composition may help to state whether a particular specimen of oil will have useful disinfecting properties or not Thus, it may be expected that oils containing one or more of the active terpene alcohols or phenols or aldehydes or ketones as their main constituents will have fairly high phenol coefficients Such was, indeed, the case, for the three oils *Cymbopogon flexuosus*, *Cymbopogon Martini* Stapf Motia, *Cymbopogon Martini*

Stapf Sofia which were observed to have the highest values in an earlier investigation (De and Subrahmanyan, *loc cit*) contained as their main constituent either geraniol or citral, both of which possess high disinfecting properties On the other hand, the occurrence of preponderating amounts of hydrocarbons with short side chains or sesquiterpenes and their derivatives will indicate that the oils containing them will have either very low or no germicidal power Thus, the Indian 'botha' grass oil was found to have a phenol coefficient of only 1 5 (De and Subrahmanyan, *loc cit*)

Although the nature and the quantity of the main constituent determines whether an essential oil will have high germicidal properties or not, yet it is not possible to evaluate, even approximately, the phenol coefficient of an oil from such a knowledge without reckoning the possible effects of the other constituents as well The latter may (*a*) possess high germicidal properties or (*b*) have low or no disinfecting property or (*c*) have properties antagonistic to that of the main constituent or (*d*) help or stimulate the growth of the putrefactive organisms which are used for the tests In the case of (*a*) the phenol coefficient will be much higher than that warranted by that of the active constituent alone in that of (*b*) it will be approximately that due to the active constituent only, the others acting merely as diluents and in those of (*c*) and (*d*) it may be either slightly or considerably lower than the one calculated from the quantity of the main constituent It should be possible in the cases of (*a*) and (*b*) to estimate approximately the germicidal values of the oils but in those of (*c*) and (*d*) which may be due largely to impurities which either distil over or collect from air, it may not be possible, except by repeated experience with the same oils, to determine the extent to which the germicidal value of any oil may be reduced by such occurrences Table I presents the phenol coefficients obtained by Penfold and Grant (*loc cit*) and De and Subrahmanyan (*loc cit*) for certain oils the more important constituents of which are known,

TABLE I

Oil	Main constituent	Other constituents	Phenol estimated	Coefficient obtained
	Percentages			
Cymbopogan Coloratus	Hydrocarbons 57	Geraniol 10, borneol 8	3	1 5
Cymbopogan flexuosus	Citral 70 to 85		12·5 to 15 2	17 0
E australiana	Cineol 62	Terpineol geraniol citral	2·1	5 0
E dives	Piperitone 52	Piperitol	4 0	8·0
Cymbopogan Martini Stapf Motia	Geraniol 75 to 95		14 to 18·1	14 0

together with the coefficients obtained by calculation assuming that the constituents (*a*) occurred as mixtures in the oils concerned, and (*b*) possessed the same phenol coefficients in the oils as when they were examined independently A comparison between the actual and the calculated values shows that, as suggested already, the former may be greater than, equal to or less than the latter

In the cases of *E australiana* and *E dives* the minor constituents had much higher disinfecting powers than the main ones and the phenol coefficients of the oils were, in consequence, higher than they were expected to be The minor constituent in *Cymbopogan Martini* Stapf Motia appears to have had no disinfecting value and acted merely as a diluent and hence the calculated and the actual values appear to have agreed On the other hand the hydrocarbons or probably the other constituents present in *Cymbopogan Coloratus* appear to have lowered the disinfecting power of the active constituents of that oil

The proportions of the active constituents of the oils are liable to vary even for specimens distilled under identical conditions in the same laboratory depending on a variety of soil and climatic factors Thus, citral in *Cymbopogan flexuosus* (lemon grass oil) is liable to vary from 70 to 85 per cent, geraniol in *Cymbopogan Martini* Stapf Motia (Palmarosa oil) from 75 to 95 per cent (De and Subrahmanyan, *loc cit*), and Safrol in *Doryphora Sassafras* from 60 to 65 per cent (Penfold and Grant, *loc cit*) This variability should make it impossible to expect the same germicidal power for all specimens of oils from the same factory unless the distillates obtained from time to time be mixed together prior to marketing

To form an approximate estimate of the germicidal value of an essential oil from its composition, it is essential to have standard values for the phenol coefficients of the different constituents The existing literature, though helpful with regard to certain of them, is highly confusing with regard to most others Thus, while Bryant (*loc cit*) by the 'Lancet' method on the one hand and Penfold and Grant (*loc cit*) and De (*loc cit*) by the Rideal-Walker method, on the other, are nearly agreed with regard to the value for citral, they are, however, at considerable variance with regard to that for geraniol (Table II)

TABLE II

	Bryant	Penfold and Grant	De
	Phenol Coefficients		
Citral	18·8	19·5	17·9
Geraniol	11·5	21·0	19·0

The points raised in the foregoing paragraphs show that the factors affecting the disinfecting power of an oil being so many and varied no more than

an impression that an oil may possess a high or low phenol coefficient may be gathered from a knowledge of the nature and proportion of its constituents However, the observation that all oils containing preponderating amounts of one or more active constituents possess, invariably, high disinfecting powers, irrespective of the method of evaluation, shows that though a precise evaluation of the germicidal property of an oil may not be possible with our present knowledge of the subject, yet indicates that the germicidal properties of essential oils are largely determined by the collective effects of their active constituents

As pointed out in the previous communication some of the cheaper Indian essential oils possess high germicidal properties and can be utilized for the manufacture of disinfectants which compare favourably in cost, non-corrosive and non-toxic properties and germicidal activity with coal-tar disinfectants The foregoing observations show that for the preparation of emulsions having identical phenol coefficients not only should oils obtained under identical conditions be used but also the same methods of emulsification, dilution and standardization should be adopted The possibility of the proportion of the main constituents varying from time to time in the different specimens of oils and thereby influencing germicidal activity should be noted and corrected by proper adjustment of dilution

The germicidal properties of emulsions of certain oils change with time, thereby suggesting that some of the active constituents probably undergo change in composition on keeping The change as in the case of *Doryphora Sassafras* (Penfold and Grant, *loc cit*) may lead to an enhanced phenol coefficient or as in that of cardamom oil (De and Subrahmanyan, *loc cit*) to a very rapid fall in germicidal power The possibility of such changes, at any rate with regard to some essential oils, should be reckoned with before admitting the accredited germicidal value even if the distribution of the constituents in the original specimens be completely known

SUMMARY

(1) The disinfecting property of an essential oil is largely determined by the nature and proportion of its active constituents

(2) Owing to various difficulties, technical and otherwise, it may not be possible to evaluate, precisely, the phenol coefficient of a disinfectant prepared out of an oil from a knowledge of its composition alone

(3) Some of the different conditions influencing the disinfecting properties of oils are discussed and suggestions made for maintaining the phenol coefficients of commercial preparations at constant values

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ON TWO INTESTINAL PROTOZOA OF AN INDIAN TURTLE

BY

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FROM time to time laboratory animals of unusual type are required by Lieut.-Colonel R. B. Lloyd, I.M.S., Imperial Serologist to the Government of India, in connection with the identification of the origin of blood-stains in criminal cases. Colonel Lloyd has very kindly arranged that, after bleeding, the perfectly fresh bodies shall immediately be made over to the Protozoology Department of the School. This material is admirable for protozoological study, since it is absolutely fresh.

In this way we received on the 18th January, 1928 the body of a Gangetic turtle (*Trionyx gangeticus*), which had been bled a few minutes previously. Examination of thick and thin films of the heart blood failed to show any protozoa, but examination of the rectal contents showed a ciliate protozoon resembling a balantidium, and a small and actively motile flagellate protozoon in abundance. Cultures were immediately put up in the 'HS&S' culture medium recommended for amœbæ by Dobell and Laidlaw (1926, p. 294), and were kept in the cool incubator at 22° to 24°C.

Examination of the cultures on the third day failed to show the ciliate protozoon, but there was a rich growth of the flagellate protozoon, and also many amœbæ of large size, loaded with grains of rice starch ingested from the culture medium. The amœba showed no contractile vacuole, whilst examination of stained preparations showed that its nuclear characters were typical of the genus *Entamœba*.

A second turtle of the same species was now obtained. Examination of its rectal contents showed (*i*) the same entamœba as before, (*ii*) the same flagellate

as before (iii) a much larger flagellate resembling a trichomastix, (iv) oocysts of what was apparently an emeria and (v) a blastocystis Cultures were again taken in the same medium as before

Examination of the first set of cultures on the fifth day showed that many of the small flagellate protozoa had encysted, whilst on the seventh day the majority of the entamœbæ had encysted We were thus able to study both organisms in fresh (cultural) material in the motile and in the encysted state Permanent preparations of both organisms were made, using cold Schaudinn's fixative and Heidenhain's non-hæmatoxylin stain For the study of the flagella of the flagellate protozoon the method advocated by Shortt (1923, p 1165) was largely relied upon

As both these organisms are of interest in connection with the literature, we may first describe each, and then discuss our findings in relationship to those of previous workers for each in turn

The Entamœba

In the fresh state in culture the size of the motile entamœba varies enormously Individuals as small as 10 μ were encountered, whilst on the other hand gigantic forms up to 60 μ stuffed with starch grains were also present The greatest diameter of fifty fixed and stained specimens was measured, this gave an extreme range of from 10 3 μ to 58 3 μ, with an average of 30 9 μ

When moving the amœba shows a definite but small volume of ectoplasm in the advancing pseudopodia In stained specimens only a mere trace of ectoplasm is seen (Plate V, figs 2 and 4) The animal is much more actively motile than *Entamœba coli* and tends to travel, but its motility is not as great as that of *Entamœba histolytica* in the fresh state Movement in general rather recalls that of a free-living amœba of *limax* type, and many individuals tend to assume a ribbon-like form as they travel Others, on the other hand, tend to assume a lobose form, and usually after the thrusting forward of an anterior pseudopodium, this is immediately followed by the protrusion of one or two pseudopodia in a lateral direction Unlike the case with *E histolytica* the amœba does not travel in the same direction continuously, but is constantly changing its direction of movement The pseudopodia are large, voluminous, dome shaped or knob-like, and are emitted with considerable rapidity

The endoplasm is markedly alveolar in character and full of spherical vacuoles (Plate V, figs 1 and 2) Starch grain are freely ingested, and big individuals loaded with starch grains are sluggish in movement (Plate V, fig 6) Often so much starch is ingested that the nucleus is pushed to one side and appears as if compressed A few drops of human blood were added to one of the cultures containing actively motile amœbæ, and specimens removed from time to time for 24 hours, no amœbæ were observed to ingest red blood corpuscles On the other hand, the animal is a voracious feeder, and its endoplasm is usually loaded with bacteria and the like, and full of digestive vacuoles

The nucleus measures about 5μ to 6μ in diameter, and is usually spherical, but sometimes a little oval in shape In the motile amœba it is barely, if at all, visible In small individuals which have not ingested much starch the ring-like outline of the nucleus can just be seen during pseudopodial activity In fixed and stained individuals the nucleus has the following characters —(*i*) there is a thin achromatinic nuclear membrane enclosing the nucleus (*ii*) On the inner aspect of this is a layer of 'peripheral chromatin' This appears to consist of granules applied to the inner aspect of the nuclear membrane, but, in all probability, this layer of peripheral chromatin is uniform in character (as is always the case in the cyst), and the beaded and granular appearance is probably due to imperfect fixation (*iii*) A karyosome of considerable volume is present, it is usually central in position, but sometimes excentric (*iv*) Between the karyosome and the peripheral chromatin is a definite linin network In some individuals there appear to be minute grains of chromatin on this linin network between the karyosome and the peripheral chromatin, but the appearances seen vary with the degree of decolourization of the stain In well-decolourized individuals the space between the karyosome and the peripheral chromatin shows nothing but traces of a linin network

Cysts —This amœba is very remarkable in the encysted state, owing to the fact that it is literally stuffed with massive chromatoid substance No cyst devoid of chromatoid substance was encountered at any time The cyst is thin walled, usually spherical, sometimes slightly ovoid Measurement of the diameter of fifty individuals in fixed and stained preparations gave an extreme range of from 7 5μ to 20 7μ, with a mean of 11 7μ, Glycogen is more usually absent than present, but when present it may be considerable in amount (Plate VI, figs 11, 18, 22, 24 and 26) The mature cyst is quadrinucleate and bears a striking resemblance to that of *E histolytica,* its massive chromatoid substance standing out in brilliant refringent relief in the fresh state In stained specimens it is seen that the chromatoid substance is massive and the cysts are loaded with bars, chunks, and masses of it Very exceptionally only is the chromatoid substance scanty in amount (Plate VI, fig 23) The nuclei are of 'histolytica type' In the early cyst the single nucleus is relatively enormous in size prior to division At the mature quadrinucleate phase the nuclei are relatively small The nuclear pattern is extremely constant There is an achromatinic nuclear membrane, on the inner aspect of this is a thin and uniform deposit of 'peripheral chromatin' The karyosome is fairly conspicuous and usually exactly central in position (In only very few individuals does the karyosome appears to be excentric in position)

Unfortunately no phases of division were encountered, either in the motile or encysted phase

Discussion

The first entamœba of a turtle to be described was *Entamœba testudinis* or *Testudo græca* by Hartmann (1910) He found this amœba in company

with a trichomonas and a balantidium He describes it as a large organism, about 50μ to 70μ in diameter, and in general closely resembling '*Entamœba tetragena*' The nucleus had a membrane with a double contour, was usually ellipsoidal and measured some 15μ by 11μ The state of the karyosome varied in different individuals in size, and in the amount of chromatin present The peripheral chromatin was abundant and arranged in the form of grains on the inner aspect of the nuclear membrane Hartmann's paper is illustrated by a good colour plate, and he concludes that *E testudinis* is intermediate in type between *E tetragena* and *E blatta* No cysts or dividing forms were observed

Alexeieff (1912) described an entamœba from the Ceylon turtle, *Nicoria trijuga*, which he considered to be identical with *E testudinis* of Hartmann He notes, however, that forms were encountered much smaller than those seen by Hartmann Taliaferro and Holmes (1924) consider that Alexeieff's amœba corresponds far more closely to their *Endamœba barreti* than to Hartmann's *Entamœba testudinis*

As is well known, Barret and Smith (1923) were the first workers to secure what was undoubtedly a successful culture *in vitro* of an entozoic amœba This was present in the intestine of the snapping turtle, *Chelydra serpentina*, and Taliaferro and Holmes (1923) in their first communication give a brief description of the organism and confirm the success of Barret and Smith in securing cultures Later, Barret and Smith (1924) gave a more detailed account of their cultural technique, and stated that they had kept cultures alive for many months Taliaferro and Holmes (1924) later gave a detailed description of this organism, which they named *Endamœba barreti*

These authors describe the motile forms of *E barreti* as ranging in general from 13μ to 23μ, with an average of 18μ The method of locomotion in general recalls that of a free-living amœba of *limax* type At times however lateral pseudopodia may be emitted The endoplasm is frequently vacuolated The nucleus is clearly visible in the fresh state In stained specimens the following structures are described —(*i*) an achromatinic nuclear membrane (*ii*) On this is a deposit of peripheral chromatin in the form of granules embedded in an achromatinic matrix on the inner aspect of the nuclear membrane (*iii*) A central or nearly central karyosome (*iv*) An achromatinic structure surrounding the karyosome which is frequently quite invisible (*v*) A definite structure, which they term a 'chromatic cloud,' surrounding the karyosome and more or less stellate in appearance, and (*vi*) a number of linin fibres which may be in the form of a network and extend from the 'chromatic cloud' to the peripheral ring They note, however, that 'the various structures described vary greatly in appearance according to the degree of differentiation in the stain'

Taliaferro and Holmes' reasons for differentiating *E barreti* from *E testudinis* of Hartmann are (*i*) the two parasites were present in different hosts, (*ii*) *E testudinis* is roughly three times the size of *E barreti*, (*iii*) the presence

of the 'chromatic cloud' within the nucleus This was not figured by Hartmann for *E testudinis*, but Taliafeiro and Holmes consider that Alexeieff's illustrations show it in his parasite, which therefore becomes synonymous with *E barreti* and not with *E testudinis*

It will be seen in general that the amœba which we have described has close resemblances to both *E testudinis* and *E barreti*, whilst it is intermediate in size between these two species We have tried in vain, however, to make out the 'chromatic cloud' surrounding the karyosome which Taliafeiro and Holmes describe and figure In deeply stained individuals it is true that one can make out appearances suggestive of the 'chromatic cloud,' and especially so in large individuals stuffed with rice starch where the nucleus has been displaced and to some extent distorted We attribute these appearances, however, to incomplete decolourization, rather than interpreting them as a definite and constant structure In the cysts the nuclei show no trace of such a chromatic cloud

It will be seen that the evidence which we have brought forward rather tends to suggest that *E testudinis* and *E barreti* are synonymous, though the matter requires further investigation Under these circumstances we refrain from suggesting any new name for the entamœba of *Trionyx gangeticus*

The flagellate protozoon

Plate VII illustrates this organism as seen in the motile phase in films stained by Shortt's method, and Plate VIII the cysts of the flagellate as seen in films fixed by cold Schaudinn's fixative and stained by Heidenhain's non-hæmatoxylin method

The body of the flagellate in the motile phase is in general pyriform, though it is subject to great variations in shape owing to its semi-amœboid activity The extremes of length in fifty fixed and stained individuals varied from 4 0μ to 15 0μ, with a mean of 8 4μ The animal is a voracious feeder, and—in common with all the *Cercomonadidæ*—ingests food particles by pseudopodial activity, the posterior half especially of the animal being crammed with bacteria, etc No cytostome can be seen

The nucleus is oval and elongated It is situated close to the anterior pole, sometimes a little laterally In non-hæmatoxylin stained preparations it is seen to be of typical vesicular character, with a large karyosome As is characteristic of the family *Cercomonadidæ*, the anterior end of the nuclear membrane is drawn out into a snout-like protrusion Presumably a tiny basal granule is situated in this position, but we have not been able to make one out

From this point three flagella arise The two anterior ones project in front of the body Of these one is invariably longer than the other, sometimes nearly double its length The longer anterior flagellum is about as long as the body of the flagellate, sometimes a little longer These anterior flagella, as studied under dark ground illumination, have a curious hooking action They

face towards one another, like two horns, and wave simultaneously towards one another

The posterior, trailing flagellum is about twice the length of the body of the flagellate, sometimes a little longer As the animal moves forward this flagellum trails behind, it is more or less adherent to the body of the flagellate as it passes backwards for the anterior two-thirds of the body, then projects free laterally and posteriorly

The movements are extremely active and rapid They are essentially of a jerky character. Only when movement slows down can the hooking action of the anterior flagella be made out

Cysts—The cysts are very small, usually spherical, but sometimes ovoid Of fifty fixed and stained specimens the diameter varied from 2·8μ to 5·0μ, with a mean diameter of 3·9μ They are literally stuffed with a brightly refractile substance which stains an intense jet black with nonhæmatoxylin Whether this substance is volutin or chromatoid substance it is difficult to say it is almost too massive to be volutin It is present in the form of blobs, bars, rods, chunks and masses In the fresh state these show up as brightly refractile spots inside the cyst

So voluminous is this deeply-staining substance within the cysts that it is difficult to make out further detail, even when the stain is well differentiated The cyst appears to be always mononucleate, the nucleus consisting of a nuclear membrane on which there is no chromatin, and a very prominent spherical karyosome Traces of glycogen may sometimes be observed inside the cyst (Plate VIII, figs 6, 15 and 17), but are not usual

Discussion

It is clear that the flagellate which we have described belongs to the family *Cercomonadidæ* and is characterized by having two anterior and one posterior, trailing, flagella It thus corresponds to the genus *Trimitus* Alexeieff (1910)

The history of this genus is a little unfortunate Diesing (1865) described a somewhat similar organism under the name of *Dicercomonas succisa*, but this was apparently a free-living form He states that it was abundant in February and March in water in which *Anodontis* was decomposing, and was also found by Perty in July in Switzerland in lake water Grassi (1879) also described a *Dicercomonas*, but unfortunately no copy of his memoir is obtainable in Calcutta

Alexeieff's organism occurred in the intestine of a marine fish, *Motella tricirrata* He describes one of the anterior flagella as being double the length of the other, and the posterior flagellum as being definitely thicker than the two anterior ones The posterior flagellum was four or five times the length of the body of the protozoon Alexeieff notes that the cytoplasm of the animal is stuffed with food particles, especially in its posterior half, the food inclusions indeed were so numerous that they rendered study of the internal structures

difficult The size of the flagellate is given as from 6μ to 8μ in length, by about 3μ in breadth

Duboscq and Grasse (1923, 1924) described what they at first regarded as a similar form from the termite *Calotermes flavicollis* in France They describe an axostyle and a small rod-like parabasal body, however, so that their organism does not correspond at all to Alexeieff's genus Later Duboscq and Grasse (1924*a*) give it as their opinion that the forms seen were merely young forms of *Trichomonas dogieli*

Chalmers and Pekkola (1919) described as *Dicercomonas soudanensis* a flagellate which they found in human fæces Finding out later, however, that *Dicercomonas* was already preoccupied, they changed the name later in the same year to *Diplocercomonas soudanensis* (1919*a*) Wenyon (1926, p 633), however, on a study of their original films, states that they were dealing with a mixture of *Trichercomonas intestinalis* and *Embadomonas intestinalis*, and that no flagellates were present having the structure of a *Dicercomonas* except some examples of *Trichercomonas* in which only two anterior flagella were visible

Alexeieff's type species is therefore at present the only valid one in the genus *Trimitus* Unfortunately Alexeieff's original description is somewhat scanty, and he did not see the cysts It is therefore difficult to determine whether the parasite which we have encountered is or is not identical with his *Trionyx gangeticus* inhabits salt water, whilst Alexeieff's parasite occurred in the intestine of a marine fish Judging from Alexeieff's measurements, the parasite which he encountered was of approximately the same size as ours On the other hand a marine fish and a river turtle are very different hosts Alexeieff named his species *Trimitus motellæ* after its host Should systematists consider that the parasite of the turtle is a different and valid species, we would follow his example and suggest the name *Trimitus trionycis* n sp for it

We are very much indebted to Colonel Lloyd for this interesting material Also to Dr Bairi Prashad of the Indian Museum, Calcutta, for very kindly looking up the literature for us, and the loan of journals from the Museum library

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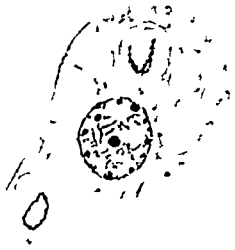

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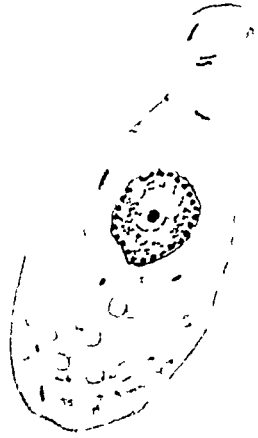

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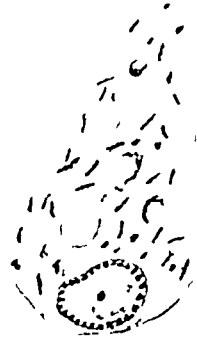

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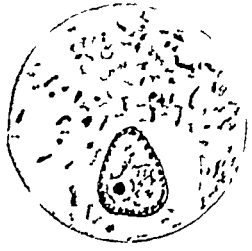

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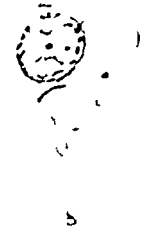

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Plate VI

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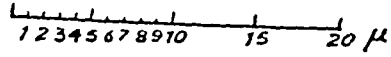


Explanation of Plates V and VI

The Entamœba of *Trionyx gangeticus* Schaudinn's fixative, iron-hæmatoxylin stain

1—8 Trophozoite forms 9—26 Cysts

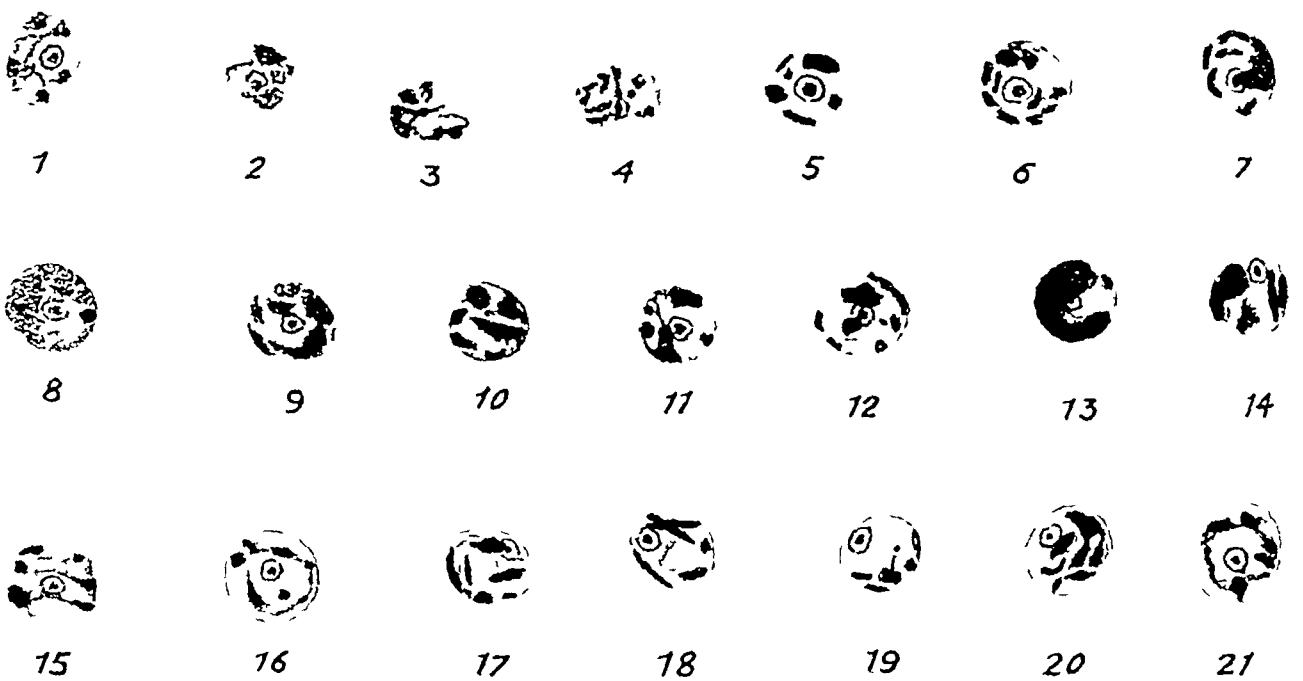
6 Giant form with three ingested starch grains

11, 18, 19, 22, 24 and 26 Cysts containing more or less glycogen

Explanation of Plate VII

The Trimitus of *Trionyx gangeticus* Motile phase
Stained by Shortt's method (fixation by osmic acid, followed by methyl alcohol, and Giemsa's stain)
5, 6, 8 and 16 Dividing forms

PLATE VIII



Explanation of Plate VIII

Cysts of the Trimitus of *Trionyx gangeticus* Schaudinn's fixative, iron-hæmatoxylin stain.

6, 15 and 17 Cysts showing traces of glycogen

ON THE DIFFERENTIATION OF *LEISHMANIA TROPICA* FROM THE PARASITE OF DERMAL LEISHMANOID

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Leishmania tropica, as it occurs in the tissues is morphologically indistinguishable from the parasite of dermal leishmanoid, though, in the case of the former, as the result of degeneration owing to secondary bacterial contamination, aberrant forms are met with

The herpetomonad forms of these two parasites also appear to be identical

In a previous communication (Das Gupta, 1927) it was shown that a culture from a dermal leishmanoid nodule, when injected intraperitoneally into a white mouse, caused visceral infection, but further experiments showed that it was also capable of producing a local lesion when injected intradermally On the other hand Row (1914, 1924) has shown that *L tropica* can induce a generalized infection when injected into the peritoneal cavity of mice

In my hands the result of experimental inoculation of mice was somewhat different, i e, *L tropica*, when injected intraperitoneally into a white mouse, produces a nodule at the site of injection, in addition to infection of the viscera No such nodule has been demonstrated so far in the case of the organism of dermal leishmanoid, when injected by the intraperitoneal route

This shows that the parasite of oriental sore has probably got a greater tendency to cause local lesions than has that of dermal leishmanoid It is thus seen that very little help can be obtained for the differentiation of these two species from the results of animal inoculation only

Attempts were also made to differentiate the species by serological tests, on the lines advocated by Noguchi (1924) Rabbits were inoculated with cultures of different leishmanias, viz, *L donovani* (of Indian origin), *L tropica*, the leishmania of dermal leishmanoid, and *L tarentolæ* of the lizard—(a strain of which was very kindly supplied through the courtesy of Dr C W Young

of the Peking Union Medical College)—giving increasing doses at six-day intervals. The sera of these animals were then tested for agglutination against homologous and heterologous strains of leishmania, but the results were completely indecisive. In fact there appeared to be no difference between the action of the 'immune' sera and that of normal rabbit serum.

Lately, my attention was drawn by Lieut.-Col. H. W. Acton, I.M.S., Director, Calcutta School of Tropical Medicine, to a demonstration at a laboratory meeting of the Royal Society of Tropical Medicine and Hygiene of the cultural characters of *Trypanosoma cruzi* and various strains of leishmania when grown on a modified Noller's blood-agar plate medium (Ray, 1929). It was shown that when *L. donovani* is grown by this method, it produces narrow streaks of growth, without lateral outgrowths, also the rate of growth is slow. On the other hand, *L. tropica*—especially a strain received from Palestine)—gave a more rapid growth and showed lateral outgrowths.

Accordingly, strains of *L. tropica* and of the leishmania of dermal leishmanoid, both isolated at about the same time—(*L. tropica* from patient Langbati on the 24th September, 1929, and the dermal leishmanoid strain from patient Roy isolated on the 2nd October, 1929)—were taken and sown on modified Noller's blood-agar plates. After 72 hours' growth the growth was gently scraped off the surface of the plates and emulsified in saline. The emulsion was standardized by first mixing equal volumes of emulsion and Sinton's fowl blood corpuscle suspension from which films were prepared, stained and counted (Sinton, 1924). This method of Sinton's, originally introduced for counting leucocytes and malaria parasites, has a very wide range of applicability, and is a most valuable laboratory procedure.

The emulsions of both species were now diluted down to the same numerical strength, and an equal number of flagellates of both species were sown on different parts of the same plate, spread as far as possible over equal areas, and incubated at 22°C.—24°C. The plates were examined daily for nine days.

It was found that the growth of *L. tropica* was far more rapid and luxuriant than that of the parasite of dermal leishmanoid, but no true lateral outgrowths, as described by Ray (1929), were seen.

It has also been found that cultures of *L. tropica* will withstand exposure to higher temperatures than those of the parasite of dermal leishmanoid. On one occasion when the cool incubator went out of order, its temperature rose very high, as a result cultures of *L. donovani* and of the parasite of dermal leishmanoid died out, and no sub-cultures could be obtained from either strain, on the other hand a culture of *L. tropica*, although the organisms appeared to have become immobile, gave a successful sub-culture on fresh medium, when incubated at the proper temperature.

It may be mentioned here that such plate cultures on a modified Noller's blood-agar medium give exceptionally rich growths, affording plentiful material for experimental purposes. The medium further has the advantage that the

growth is free from the debris present in the water of condensation of NNN medium, and it is very suitable for preparing stained specimens and for teaching purposes It also produces heavy infection in experimental animals Thus Plate IX, fig 3 is a microphotograph of a spleen smear from a white mouse which received an intraperitoneal injection of such a culture of the parasite of dermal leishmanoid, and died from leishmania infection on the 34th day

I wish to express my indebtedness to my chief, Col R Knowles, I M S , for his help and guidance in this work

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NOTE ON THE USE OF AMMONIUM MOLYBDATE IN KUTTNER AND COHEN'S METHOD OF MICRO-ESTIMATION OF PHOSPHATES

BY

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In working with Kuttner and Cohen's method (1927) for the estimation of small quantities of phosphates in urinary stones, difficulty was experienced because of the frequent occurrence of a blue coloration in blanks containing no phosphate whatsoever. These authors themselves have found that their method worked within very narrow limits. Thus, the optimum acidity of sulphuric acid for reduction of the phosphomolybdic acid lies in a zone between 0·9 and 1·05 normality, and any variation from this range produces either a decrease or an increase of colour. Again, the optimum concentration of sodium molybdate in the final mixture lies in the very narrow zone between 0·73 to 0·75 per cent., concentrations below this zone effect a decrease in colour-production while those above it increase colour-production due to the reduction of molybdate itself. Lastly, the optimum concentration of stannous chloride lies in the zone between 0·020 and 0·022 per cent., and stronger solutions reduce molybdic acid as well as phosphomolybdic acid. Thus it is seen that the three principal reagents used in the method, viz., sulphuric acid, sodium molybdate and stannous chloride, would all have an adverse effect on the accuracy of the method, if used in other than their individual optimum concentrations.

The frequent use of ammonium molybdate in analytical laboratories and the ease with which it is obtained in a high state of purity, combined with the difficulty of obtaining sodium molybdate, suggested the substitution of an equivalent amount of ammonium molybdate in Kuttner and Cohen's method

Allowing a 10 per cent error for colorimetric estimations, it can be seen from the above table that the optimum zone for the sulphuric acid in the case of ammonium molybdate lies between 0 7 N and 1 1 N Any change in the normality especially on the lower side of 1 0 N would not so vitiate the accuracy of the results as it would if sodium molybdate were used In the blanks for ammonium molybdate, only one with a 0 5 normality gave an immediate distinct blue coloration, this shows that in very weak acid solutions, even the molybdic acid is reduced by the stannous chloride But in blanks with ammonium molybdate having concentrations of 0 7 N and over there is either no colour produced within one hour or at best only a slight yellowish tinge This is not so when sodium molybdate is used, for in every one of the blanks there was an appreciable blue coloration, which ranged from 43 to 90 per cent of the colour that would be developed under similar conditions with 0 1 mg of P_2O_5

TABLE II

Experiment 2

Normality of sulphuric acid and percentage of stannous chloride constant, but percentage of molybdates varying

Number	With ammonium molybdate			With sodium molybdate		
	Percentage of molybdate	Percentage colour production	Percentage colour production in blanks (without phosphate)	Percentage of molybdate	Percentage colour production	Percentage colour production in blanks (without phosphate)
1	0·1	0		0 50	106·6	42 8
2	0·2	28·1		0 60	103 0	Trace *
3	0 3	84 5		0 70	107 6	14 3 *
4	0 4	100 0	Only a light yellowish tinge after over half an hour	0 73	99 7	14 3 *
5	0 5	101 2		0·75	100 0	17 9
6	0 6	100 4		0 80	117 4	39 4
7	0·7	100·4		0·90	130 2	46 5
8	0·8	102·0				

*In the blue colour obtained, there was a greenish tinge which rendered the colorimetric comparisons difficult and not very trustworthy

It is evident from the above table that the optimum zone for the concentration of ammonium molybdate lies over a wide range from 0 4 to 0 8 per cent As nothing is gained by increasing unnecessarily the ammonium molybdate content, it is kept at 0 4 per cent This result is in contrast to that

obtained by Kuttner and Cohen, where a slight deviation from the very narrow optimum zone of 0 73 to 0 75 per cent produces a marked increase or decrease in colour production according as the percentage of sodium molybdate is increased or decreased Here then lies the special advantage of ammonium molybdate over sodium molybdate

It is to be noted that while the blanks with ammonium molybdate were free from the blue coloration, those with sodium molybdate were distinctly blue, the depth of colour varying from about 14 to 46 per cent of that which would be developed under similar conditions with 0 1 mg P_2O_5

TABLE III

Experiment 3

Normality of sulphuric acid and percentages of molybdates constant, but percentage of stannous chloride varying

Number	With ammonium molybdate			With sodium molybdate		
	Percentage stannous chloride	Percentage colour production	Percentage colour production in blanks (without phosphate)	Percentage stannous chloride	Percentage colour production	Percentage colour production in blanks (without phosphate)
1	0·0025	62 5				
2	0 005	94 0		0·005	80·7	23·2
3	0 010	98·0	There was no production of the blue colour characteristic of the phosphate Even several hours standing did not result in the development of the blue colour	0·010	92·8	35 7
4	0·015	100 4				
5	0 020	100 0		0 020	100·0	39 3 *
6	0 025	98·5		0 022	99 8	39 3 *
7	0·030	99 5 *		0 025	103 6	35 7 *
8	0·040	87 0 *		0 030	111 0	26·7 *
9	0 080	88·5 *		0·040	112·2	26·7 *

* There was also a greenish tinge which rendered the colorimetric comparisons difficult and not very reliable

It is seen from Table III that the optimum zone for stannous chloride in the case of ammonium molybdate is spread out over a much wider range, viz, 0 005 to 0 025 per cent, than in Kuttner and Cohen's method With increasing concentrations of stannous chloride, especially 0 03 per cent and over (Numbers 7, 8 and 9), the characteristic blue colour was masked by a greenish tinge, rendering colorimetric estimations difficult and not very reliable The production of a blue colour in blanks was, as observed before, noticed only when sodium molybdate was used, while the blanks performed with ammonium molybdate were free from it

Mean colour production in blanks with sodium molybdate

Taking the colour produced with 0·1 mg. of P_2O_5 under Kuttner and Cohen's optima for the reacting substances, as equivalent to 100, the following values were obtained in four blanks performed under similar conditions: 43·0, 17·9, 39·3 and 16·6. These represent a mean colour production of 36·7.

Ammonium molybdate versus sodium molybdate

In the experiments designed to ascertain the relative intensities of the blue colours developed with ammonium molybdate and sodium molybdate, the colour production with ammonium molybdate was only about 72 per cent. of that obtained with the sodium salt. This is not to be taken as indicating a less intense colour with ammonium molybdate, for while the blank with ammonium molybdate was free from blue colour that with sodium molybdate was equivalent to 16·6 per cent. of the colour obtained with 0·1 mg. P_2O_5. On the other hand, ammonium molybdate would appear to produce a deeper colour, if due allowance be made for the blank.

Discussion

The necessity of putting up blanks in the micro-estimation of phosphates can hardly be overestimated, since a distinct blue coloration, characteristic of the phosphate, was obtained in almost every blank done with sodium molybdate. Failure to do blanks may very likely lead to the detection and estimation of phosphate where none is actually present. But a considerably greater degree of freedom from this fallacy can be got by the use of ammonium molybdate in place of the sodium salt, though even then it is always safer to perform blanks. It is not possible to infer from Kuttner and Cohen's paper whether blanks were done, or having been done whether they were inadvertently omitted in the text of the paper.

It might be argued that the development of the blue colour in blanks containing sodium molybdate may be due to an impurity of phosphate in the molybdate. The substance used in the present investigation was obtained from the British Drug House. Boiling about half a gram of the substance with concentrated nitric acid did not give an yellow precipitate of phosphomolybdate. As the sodium molybdate used here was not of the specification given by Kuttner and Cohen, viz., Kahlbaum's 'Zur Analyse,' it is not possible at the present stage to say definitely whether there will be similar colour production in blanks or not. Even assuming that there is no colour production, ammonium molybdate is to be preferred. Its easy availability in a high state of purity, its relative cheapness, the wide range of concentrations within which it can be employed and the almost complete freedom from colour in the blanks are all points in favour of using ammonium molybdate in the micro-estimations of phosphate.

SUMMARY AND CONCLUSIONS

A modification of the Kuttner and Cohen's method of micro-estimation of phosphates has been effected by the substitution of the more common ammonium molybdate for the relatively rarer sodium molybdate This substitution confers on the method many advantages

REFERENCE

KUTTNER and COHEN (1927) *Jour Biol Chem*, 75, p 517

BACTERIOPHAGE IN BACILLARY DYSENTERY AND CHOLERA

BY

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BACTERIOPHAGE IN BACILLARY DYSENTERY

THE present series of observations on the incidence of bacteriophage in bacillary dysentery and the effects of treatment with a therapeutic phage was carried out at the Rangoon General Hospital during 1928 and 1929 mainly during the monsoon seasons when dysentery was epidemic The object of the work was to observe the natural incidence of phage and the effects of phage treatment in fresh acute cases of pure bacillary dysentery, and especially in cases dealt with before the occurrence of severe intestinal lesions such as ulceration and necrosis It was considered that such cases would, if the therapeutic administration of phage was capable of proving effective, show the most definite results Cases were selected which were of short duration on admission and which showed the characteristic cellular exudate of bacillary dysentery of the acute stage, and only those included from which the causative organism was isolated The class of patient entering the Rangoon General Hospital, and the conditions under which they entered, made it by no means easy to obtain a large number of suitable cases for our observations Many came late with established ulceration of the colon and even in cases which showed typical bacillary dysentery and from which the organism was isolated a later appearance of *E histolytica* in the stools showed the presence of double infection Such cases were excluded from the final series The difficulty in obtaining suitable cases conforming to our requirements will be realized when it is noted that in 1929 out of 878 cases whose stools were examined only 24 were found suitable for continued observation In the majority of cases the exudate was atypical and a first culture negative and these were not proceeded with One hundred and five were amœbic dysentery The above explanation accounts for

TABLE I

Shiga dysentery—1928 series

Incidence of bacteriophage in stools as tested against stock laboratory strain

Days from admission	Controls							
	Case Number							
	6	12	16	18	20	22	24	26
1	++	++	+	++	+	+	0	0
2	+++	—	+++	++	+++	+++	+	+
3	+	+++	+++	+++	+++	+++	+	+++
4	0	++	+++	+	+++	+++	+	+++
5	0	—	+++	+	0	+++	+	+++
6	0	—	+++	++	—	++		+++
7	—	—	—	++	+++	+++		+++
8	—	—	—		+++	+++		+++
9	0		++		+++	+++		+
10						+++		+++
11								+++
12								0
13								+
14								+
15								+

| | | |

TABLE I—*concld*

Days from admission	Treatld cases													
	Case Number													
	1	5	7	11	15	17	19	21	23	27	28	29	30	31
8	+++	—			+++			+++		+++	+++	+++		+ +
9	+++				+++	•	+++			+++	+++			
10	+++				+++						+++			
11	+++													
12	+++													
13	+++													
14	—													
15	+++								•					
Result	Dis-charged	Dis-charged	Dis-charged	Died dysen-tery	Dis-charged	Dis-charged	Dis-charged	Dis-charged	Dis-charged	Died	Dis-charged	Dis-charged	Dis-charged	Died
Days in hospital to discharge or death	15	21	12	6	39	6	10	8	5	9	13	11	11	21
Number of days before clear of microscopical evidence of dysentery	11	5	4	6	10	5	8	6	4	9	9	7	8	12

Cause of death
No 12 tuberculosis of lungs
No 26 dysentery
No 11 dysentery
No 27 dysentery
No 31 debility

TABLE II

Shiga dysentery—1929 series

Incidence of bacteriophage in stools as tested against stock laboratory strain and own organism

Days from admission	Controls											
	Case Number											
	1		15		16		19		21		26	
	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stock	Own
1	++	+	++	++	+++	+++	0	+++	0	0	0	++
2	0	+	—	—	—	—	+++	++	0	+++	+	++
3	0	++	++	+++	+++	+++	0	+++	+++	+++	0	+
4	0	0	++	+++	+++	++	—	—	+	+++	0	++
5			+++	+++	—	—	+++	+++	—	—	—	—
6			+	+++	++	+++	—	—	—	—	+++	+++
7			+++	++++	+++	+++	+++	0	0	++++	+++	+++
8			++	+++	+++	++	++	++	+++	+++	+++	++
9			—	—	—	—	—	—			0	+++
10			—	—	—	—	++	+++			—	—
11			+	+++	—	—	—	—			++	++
12			+++	++	0	+++	—	—				
13			+	+++	0	++	—	—				

TABLE II—*contd*

Days from admission	Controls											
	Case Number											
	1		15		16		19		21		26	
	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stock	Own
14			+++	+++	++	++	0	++				
15			+++	++	0	0						
16			—	—	—	—						
17			0	+++	0	++						
18			++	+++	++	+++						
19			+++	+++	0	++						
20			+	0								
21			0	+++								
Result	Discharged		Discharged		Discharged		Discharged		Discharged		Discharged	
Days in hospital to discharge or death	4		21		36		11		8		11	
Number of days before clear of microscopical evidence of dysentery	4		19		16		9		8		11	

Days from admission	Treated cases											
	Case Number											
	2		3		7		11		12		13	
	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stock	Own
1	++	++++	+++	+++	+++	+++	++	+++	+++	+++	+++	++++
2	++	+++	+++	++++	+++	++++	+	+++	—	—	+++	++++
3	0	+++	+++	+++	+++	++++	++++	++++	+++	+++	+++	++++
4	++	++	+++	++	+++	++++	+++	++++	++—	+++	+++	+++
5	—	—	—	—	+++	+++	—	—	+++	++++	+++	+++
6	++	+	—	—	—	—	+++	++++	+++	+++	+++	++
7			+++	+++	+++	+++	+++	++++	++	+++	0	+++
8			++	0			+++	+++	++	++++	++	+++
9			0	+			++	++++	—	—	++	+++
10			++	+			+++	++++	++	+++	—	—
11							+++	++	+++	+++	0	+++
12							—	—			+++	++++
13							+++	++			++	+++
14							+++	+++			+++	+++
15							0	++++			—	—
16							+++	+++			—	—
17							+++	+++			++	+++
18							++++	+++			+++	+++

TABLE II—*concld*

Days from admission	Treated cases											
	Case Number											
	2		3		7		11		12		13	
	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stock	Own
19							—	—			—	—
20							++	+++			0	+-' +
21							++	+++			—	—
22											+	+++
23											—	—
24											—	—
25										•	+++	+++
26											+++	+++
27	•			•							0	+++
28											+++	0
Result	Discharged		Discharged		Discharged		Discharged		Discharged		Discharged	
Days in hospital to discharge or death	6		10		7		30		17		43	
Number of days before clear of microscopical evidence of dysentery	6		4		4		17		5		26	

TABLE III

Flexner dysentery—1929 series

Incidence of bacteriophage in stools as tested against stock laboratory strain and own organism

Days from admission	Controls											
	Case Number											
	4		21		22		23		25		27	
	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stock	Own
1	+++	+++	++	++	0	++	0	+	0	++	+++	++
2	+++	+++	—	—	0	++	+++	+++	++	0	+++	0
3	—	—	+++	+	0	+++	0	+++	+	+	+++	++
4	—	—	++	++	—	—	++	++	—	—	—	—
5	—	—	+	+++	++	++	—	—	0	+++	0	++
6	—	—	0	++	+	++	0	0	+++	++	+++	0
7	+++	++	+++	0	0	0	0	0	+	++	+++	0
8			—	—	0	0			0	0	+++	+++
9			—	—	0	++					—	—
10			0	0							0	0
11			++	0							—	—

TABLE III—*concld*

Date from admission	CONTROLS											
	Case Number											
	4		21		22		23		25		27	
	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stock	Own
12			+++	+++								
13			0	0								
Result	Discharged		Died		Died		Discharged		Discharged		Died	
Days in hospital to discharge or death	37		13		19		30		20		11	
Number of days before clear of microscopical evidence of dysentery	7		13		8		6		6		11	

Days from admission	TREATED CASES											
	Case Number											
	8		9		10		14		17		20	
	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stock	Own
1	++	++	+++	++	++	++++	++	++++	+++	++	++++	+++
2	—	—	++	++++	++	0	++	+++	0	+++	—	—
3	++	++	++	++++	—	—	++	+++	+++	0	+++	+++
4	++++	+++	+++	+++	0	++++	+++	++++	++	++	+++	+++

5	—	—	—	—	—	—	+++	++++	0	++	+++	++
6	—	—	+++	++++	+	0	+++	+++	—	—	—	—
7	++	++++			+++	+++	+++	++++	++	++	+++	+++
8	+++	++++					+++	+++	++	++	+++	+++
9	+++	+++					+++	+++				
10	+++	++++					—	—				
11	+++	++++					—	—				
12	—	—					—	—				
13	—	—					+++	+++				
14	+++	++++										
15	++	+++										
16	+++	+++										
Result	Discharged		Discharged		Discharged		Discharged		Died		Discharged	
Days in hospital to discharge or death	16		6		8		31		8		30	
Number of days before clear of microscopical evidence of dysentery	14		4		6		6		8		5	

Cause of death

No 21 dysentery and chronic interstitial nephritis
No 22 tuberculosis of lungs
No 27 dysentery
No 17 dysentery

TABLE IV

Showing phage activity of stool filtrates of 'control' cases dying within 24 hours of admission against stock vibrio and own organism

Days from admission	Case 17		Case 20		Case 31		Case 34		Case 44		Case 51		Case 63		Case 64		Case 65		Case 71	
	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stock	Own
1st	0	0	++	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0
2nd	Died		Died		Died		Died		Died		Died		Died		Died		0	0	Died	
3rd																	Died			

TABLE V

Showing phage activity of stool filtrates of 'treated' cases dying within 24 hours of admission against stock vibrio and own organism (Stools taken before administration of therapeutic phage)

Days from admission	Case 2		Case 4		Case 11		Case 15		Case 17		Case 26		Case 30	
	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stock	Own
1st	0	—	0	—	+	+	+	+	0	0	+++	—	0	0
2nd	Died		Died		Died		Died		Died		Died		Died	

TABLE VI

Showing phage activity of stool filtrates of 'control' cases living more than 24 hours after admission against stock vibrio and own organism

Days from admission	Case 3		Case 6		Case 10		Case 13		Case 14		Case 15		Case 53		Case 68		Case 71	
	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stool	Own	Stool	Own	Stock	Own
1st	0	—	0	0	—	—	—	—	0	—	—	—	—	—	—	—	0	0
2nd	++	+	0	0	0	0	0	0	0	0	++	0	0	0	0	0	0	0
3rd	+	+	++	+++	—	—	0	0	0	0	0	0	—	—	0	0	++	0
4th	+	+	++	—	—	—	0	0	0	0	0	0	0	0	0	0	—	—
5th	+	—	—	—	0	0	0	0	0	0	—	—	—	—	0	0	+	0
6th	+	—	—	—	+++	0	++	+	0	0	—	—	—	—	+	0	—	—
7th	+	—	—	—	—	—	0	0	—	—	—	—	0	0	—	—	—	—
8th	—	—	—	—	+	0	0	0	0	0	—	—	0	0	0	0	—	—
9th	—	—	+++	++++	0	0	—	—	—	—	0	+	0	0	—	—	0	0
10th	—	—	0	0	0	0	—	—	0	0	0	0	0	0	—	—	0	0
11th	—	—	0	0	0	0	—	—	0	0	0	0	+++	0	0	0	0	0
12th	—	—	—	—	Discharged		—	—	0	0	0	0	Discharged		—	—	0	0
13th	Discharged		—	—			—	—	Discharged		0	0			0	0	0	0
14th			—	—			++	0			0	0			Discharged		Discharged	
			Discharged 22nd day				Discharged 29th day				Discharged 23rd day							

Table VII

Showing phage activity of stool filtrates of 'treated' cases living more than 24 hours after admission against stock vibrio and own organism (Stools on first day were taken before administration of therapeutic phage) The date of cessation of phage treatment is underlined

Days from admission	Case 7		Case 19		Case 32		Case 49		Case 61		Case 66		Case 73	
	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stock	Own	Stock	Own
1st	—	—	+++	0	0	—	0	0	0	0	0	0	—	—
2nd	—	0	—	—	0	0	0	+	0	0	0	0	+++	0
3rd	+++	0	+++	0	—	—	0	+	+++	0	0	0	+++	0
4th	++++	—	+++	0	+++	0	—	—	+++	0	+	0	++	0
5th	0	0	—	—	+++	0	—	—	+++	0	0	0	+	0
6th	+	0	+++	0	+++	0	—	—	0	0	0	+	—	0
7th	—	—	+	0	+	0	—	—	0	0	0	0	—	—
8th	—	—	++	0	0	0	+	+	0	0	0	0	—	—
9th	—	—	—	—	+++	0	+++	0	—	—	—	—	—	—
10th	—	—	—	—	Died		0	++	—	—	—	0	—	—
11th	—	—	—	—			0	0	0	0	0	0	0	0
12th	++	0					0	0	0	0	0	0	—	—
13th							0	0	0	0	0	—	0	0
14th	0	0					0	0	Discharged		—	0	0	0
15th							Discharged				0		+++	0
	Discharged 26th day		Discharged 19th day		Discharged 17th day						Discharged 19th day		Discharged	

THE PATHOLOGY OF CHRONIC COLITIS IN THE TROPICS *

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THE term 'colitis' has been used clinically for various obscure affections of the large intestine characterized by diarrhœa or by the passage of mucus or even blood in the motions The underlying pathological condition has not been clearly described The term itself presupposes an inflammatory affection of the colon Varying grades are met with from mere superficial catarrh of the mucous membrane to actual ulceration and even total destruction and sloughing of the colon Descriptions of the morbid anatomy of the lesions met with naturally vary in such a vague group It must be understood that the condition called muco-membranous colic characterized by constipation, attacks of colicky pain and the passage of membranes is not included under 'colitis,' in agreement with the view that the condition is due to a hyper-secretion of vagal origin very similar in its mechanism to asthma

'Enteritis' is a similar term applied to an acute or chronic catarrhal affection of the bowel or more particularly the large intestines The term 'follicular' ulceration connotes a type of ulceration confined to the lymph follicles of the small and also of the large intestine 'Stercoral ulceration' is also another term that has been used to denote a type of ulceration thought to be due to the presence of hard scybalæ 'Diphtheritic' colitis, 'croupous' enteritis are terms that were commonly used to designate that type of acute inflammation of the colon characterized by the presence of a false membrane of fibrin and inflammatory cells The term 'ulcerative colitis' has been used to designate an affection of obscure ætiology but here in India at least most of the cases labelled as such show at autopsy well-marked features of dysenteric ulceration The term 'dysentery' itself is rather loosely applied to ulcerative affections of the colon due to infection either with the *Entamœba histolytica*

* A paper read at the Indian Science Congress, 1930

or with bacilli belonging to the Shiga or Flexner-Y types 'Gangrenous colitis' is a name given to a type of severe ulcerative colitis characterized by the presence of dirty grayish black sloughs

Colitis has been attributed to various causes such as chronic constipation, infection from various sources such as the food, mouth, appendix, to mechanical irritation from kinks and stenosis, to intestinal parasites, etc Excretion of uric acid through the colon in gouty conditions giving rise to a gouty colitis has also been described

A study of the post-mortem records and material obtained from about 800 cases of various types of inflammatory and ulcerative affections of the bowel has shown the importance of what may be called the 'dysenteric' factor in all these affections

For a proper appreciation of this a description of types of dysenteric lesions we have met with are of interest

A study of post-mortem material in bacillary dysentery shows one great similarity in that the lesions are always inflammatory It is not sufficiently recognized, however, that the reaction varies to a great extent with the type and virulence of the infecting organisms

Early lesions in severe cases are mostly in the crests of the folds of the mucous membrane and consist of a speck of necrosis covered by whitish gray slough—a diphtheritic reaction This is surrounded by an area of acute congestion and hæmorrhage into the mucous membrane However, it is quite common to meet with early lesions in which the *hæmorrhagic reaction* is more predominant and the diphtheritic reaction is not so marked, so that the lesions found post-mortem are small serpigenous ulcers surrounded by hæmorrhage extending along the crests of the folds of the mucosa, the ulcers being mostly superficial while the whole mucous membrane shows intense congestion and points of hæmorrhage Sometimes specks of hæmorrhage with a central area of necrosis are the first lesions that are noticed Very often the whole of the sigmoid and the descending colon are covered with large irregular serpigenous ulcers with intense engorgement of the surrounding mucosa The ulcers are covered with dirty yellow sloughs extending in sheets, an appearance corresponding to the diphtheritic colitis of old writers In very acute infections a 'gangrenous' process may supervene, the whole mucous membrane showing intense infiltration with inflammatory cells It is to be noted that chronic cases of bacillary dysentery do not present this typical diphtheritic or hæmorrhagic reaction Indeed in the great majority of cases late lesions can only be made out by an examination of sections which show the type of reaction The lesions are invariably inflammatory even in chronic cases Proliferative changes in the mucous membrane are quite common, giving rise to polypoid elevations from the presence of adenomatous masses or cysts in the glands, *colitis polyposa* On the other hand atrophy of the bowel may supervene from extensive ulceration of the mucosa and the end result may be a pale or pigmented and attenuated intestine, which microscopically exhibits very little

glandular tissue and in which all the coats show atrophy The commonest type, however, is a thickened bowel presenting what may be called a 'moss covered' appearance of the mucous membrane with irregular sears in places This is due to the superficial ulceration of the villi so that the uniform smooth texture of the mucosa is lost It must be recognized that the commonest 'follicular' ulcer that is met with here is in infections with the dysentery group and such ulcers form the basis of the so-called 'granular colitis' of old writers So-called 'stercoral' ulcers identical in every respect with ulcers in the neighbourhood of strictures and kinks are met with in chronic infections with the dysentery bacilli The question arises whether these types of ulcers are really dysenteric in nature Histologically 'stercoral' ulcerations are indistinguishable from chronic dysenteric ulcers

When we turn to amœbic dysentery we find a different reaction of the colon, not inflammatory but degenerative and necrotic This is obvious from a study of amœbic lesions There is a necrosis of tissue which involves the mucosa and submucosa and extends down to the muscle coat causing extensive and deep ulcers Early lesions corresponding to the first type of Bartlett (1917), where there are tiny pin-point nodules without any breach of surface, have not been met with in autopsies Minute nodules with yellow ulcerated margins corresponding to Bartlett's second type have been met with The usual type of amœbic lesion is a large irregular ulcer covered by gray and necrotic sloughs and with undermined edges, the surrounding mucosa showing a boggy appearance Bartlett's classification of five types of amœbic lesions are not borne out by our records In chronic cases some ulcers have clear cut margins and a punched-out appearance, while others have a honey-combed appearance The wall of the intestine is usually thinned out and atrophied In hyper-acute infections the mucous membrane is extensively ulcerated and a gangrenous process is sometimes present The ulcers have at their edges black or gray tags of necrosed mucous membrane and the base of the ulcers themselves are dark, discoloured and friable The colon may be very much thinned out and friable and perforations sometimes occur Here we have a different type of reaction with destruction of tissue Very little inflammatory changes are observed in uncomplicated infections with *E histolytica* 'Mixed' infections, however, occur in which the necrosis of tissue caused by the *Entamœba histolytica* and inflammatory lesions, probably due to dysentery organisms, are both present

What are the lesions met with in chronic colitis and chronic diarrhœa? Mummery (1910) recognizes three pathological types, (1) hypertrophic colitis where there is glandular proliferation, (2) granular colitis where there is follicular ulceration and inflammation, and (3) chronic catarrhal colitis Herschall and Abrahams recognize besides these (4) an atrophic from which is the late result of a chronic catarrh An analysis of the post-mortem findings of 40 cases of chronic diarrhœa described variously as chronic colitis, enterocolitis, tubercular diarrhœa, etc, have showed lesions indistinguishable histologically from that

of chronic bacillary dysentery in 18 cases and from amœbic dysentery in 6 cases In the remaining 16 cases the only condition met with was an atrophied intestine showing atrophy of the glands, atrophy of the mucous membrane and even of the muscle coat Whether these are lesions met with as a result of previous dysenteric infection, or whether they represent an atrophied state of the intestine as a result of chronic inanition or possibly vitamin deficiency, or whether they represent atypical cases of sprue, it is not possible to say at this stage It seems probable, however, from a study of post-mortem material that a large number of these cases of chronic diarrhœa are really the result of chronic infection with dysentery bacilli or *Entamœba histolytica*

It might be asked why it is that cases of chronic colitis do not give definite evidence on culture of dysenteric infections Bacteriologists have pointed out that dysentery organisms survive in the gut only in the very earliest stages of infection and that in mild types dysentery organisms may cause progressive ulceration of the gut and that cultures even in typical cases are most difficult to obtain unless they be undertaken sufficiently early before fœcal organisms overgrow the dysentery group Thus Manson-Bahr and Gregg (1925) have pointed out instances of cases of bacillary dysentery of a mild type resulting in progressive ulceration of the bowel in which the specific dysentery bacilli play but the initial rôle Probably the acute infection causes the destruction of the mucosa and the other organisms set up a chronic catarrh Ledingham has pointed out on bacteriological evidence that the term 'colitis' has been wrongly applied to conditions the dysenteric nature of which was not far to seek, and he brings forward strong evidence to prove the importance of *B dysenteriæ* in the ætiology of primary simple diarrhœas He calls the condition a simple enteritis of Flexner origin and also adds that in essence these Flexner diarrhœas are abortive dysenteries

When we turn to *Entamœba histolytica* infections Wenyon and O'Connor have pointed out that this infection is very often latent and that many have mild attacks of diarrhœa and only some have definite dysentery This is borne out by Ledingham's observations on cases from 1922-24, which point to the fact that *E histolytica* is more often found in cases of diarrhœa than in dysentery

It might be argued that in the absence of the demonstration of the infecting agents we have no real evidence as to the dysenteric nature of these chronic diarrhœas, but my contention is that the morphological appearances of the lesions show striking resemblances to dysenteric lesions either caused by the dysentery bacilli or *Entamœba histolytica* Bacteriological evidence of this as I have pointed out is extremely difficult, if not impracticable, to obtain

Summary

1 The different types of dysenteric lesions are described from a study of material and records from 800 autopsies

2 It is put forward that about 60 per cent of fatal cases of 'chronic' colitis or 'chronic diarrhœa' of indefinite origin show morphologically strong resemblance to the lesions of chronic dysentery

3 Some cases show an atrophied state of all the coats of the large and small intestine, the exact cause of which has not been determined

REFERENCES

Mummery (1910)	'Diseases of the Colon,' p 137
Herschell and Abraham (1911)	Chronic colitis,' p 11
Manson-Bahr, P and Greig, A L (1925)	*Brit Jour Surgery*, p 711
Ledingham, J C	Official History of the Great War' (Pathology)
Wenyon and O'Connor	*Cited by* Ledingham *Ibid*
Bartlett (1917)	*Quart Jour Med*, XI, p 349

Explanation of Plate X

Bacillary dysentery

P 464 *Slide*

Small intestine showing early lesions and small superficial serpigenus ulcers with hæmorrhages

Microscopically shows infiltration with polymorphs and round cells and submucous hæmorrhages and superficial ulceration

PLATE XI

Explanation of Plate XI

Chronic colitis

P M 2231
13-12-28

There are healed ulcers leaving the submucosa bare in places The mucous membrane has a 'moss covered' appearance from superficial ulceration There is some thickening and stenosis of the gut

Slide P 425 shows old hæmorrhages, irregular ulceration of the mucosa, œdema and thickening of the submucosa and infiltration with polymorphs ? Chronic bacillary dysentery

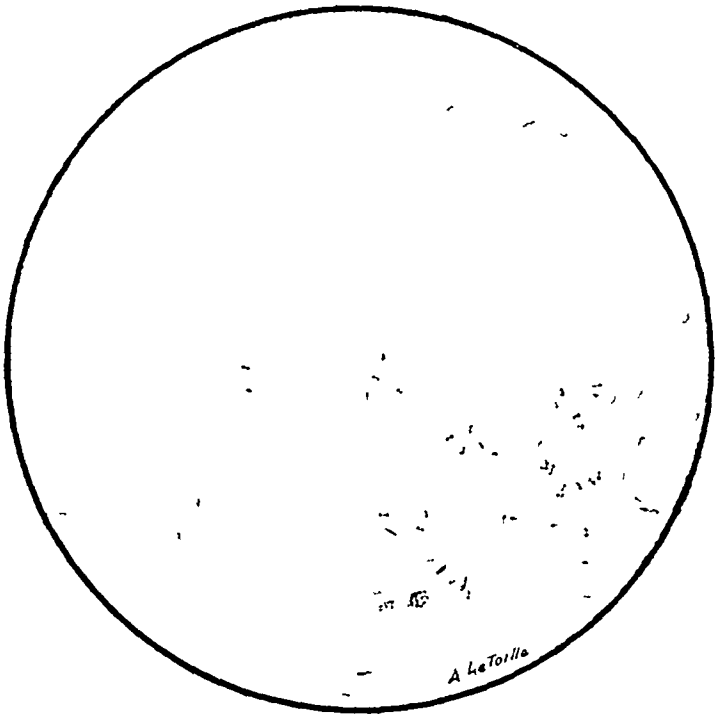
Explanation of Plate XII

Chronic colitis

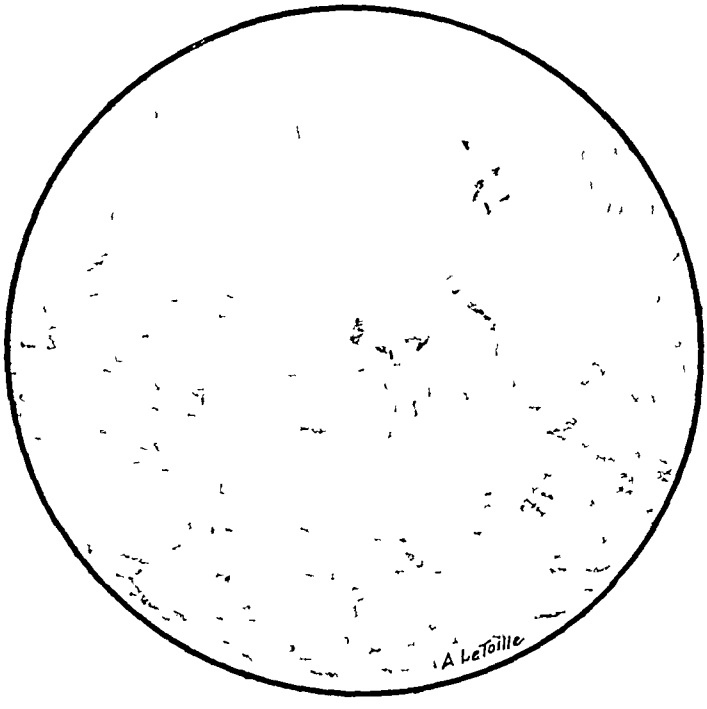
Slide P 1327

Shows necrosis of the mucous membrane in places and infiltration with polymorphs and round cells. There is marked infiltration of the submucosa with round cells, polymorphs and a few macrophage cells. The vessels of the submucosa show marked congestion and there are a few hæmorrhages. ? Chronic bacillary dysentery.

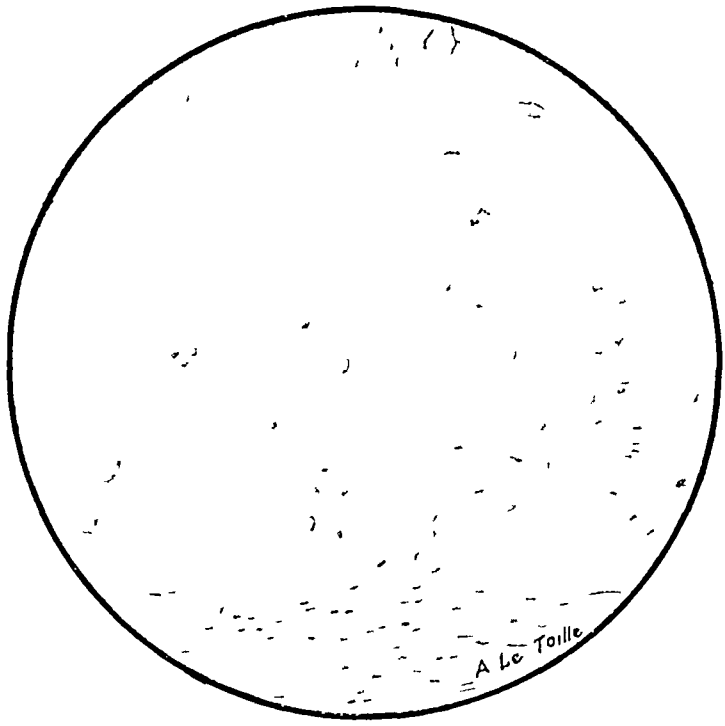
PLATE XII



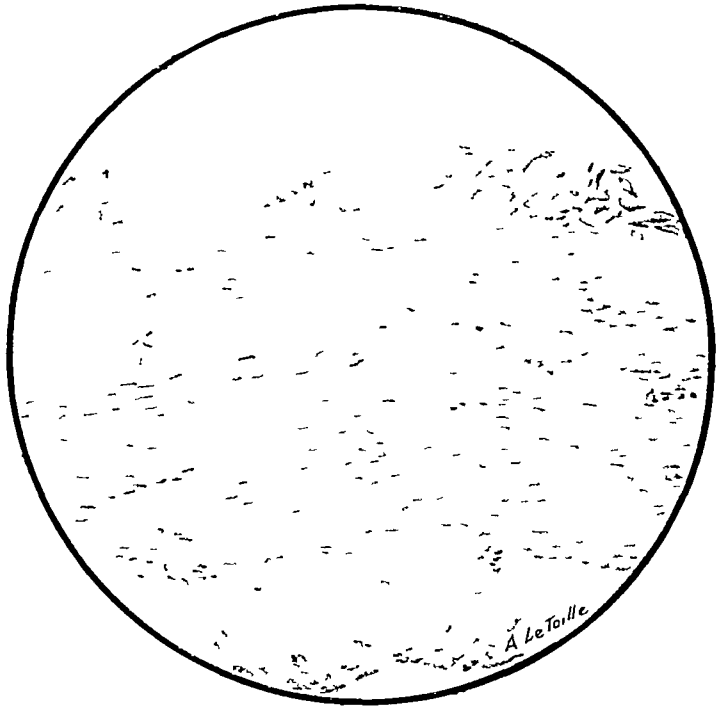
Slide 5351 Acute bacillary dysentery



Slide P 425 Chronic colitis showing similar histologic reaction



Slide P 931 Acute amœbic dysentery



Slide P 1248 Chronic colitis, atrophy and ulceration of the mucosa

A SHORT NOTE ON THE AGGLUTINOGENETIC POWERS OF THE 'ROUGH' AND 'SMOOTH' VARIANTS OF *B TYPHOSUS* AND THEIR MUTUAL IMMUNOLOGICAL RELATIONSHIP

Part II.

BY

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VACCINES made from the 'rough' strains of micro-organisms have been proved to be inferior, for prophylactic purposes, to those made from the 'smooth' strains, although a few workers have assigned protective values to the former against infection by the smooth (virulent) strains

Arkwright (1927) carried out a series of experiments with guinea-pigs immunizing them with the rough and smooth strains of *B typhosus* and tested the protection afforded by each against intraperitoneal lethal doses of the smooth strain Weber (1927) has shown that avirulent strains (rough) of *B typhosus* did not make efficient prophylactic vaccines Rowland (1914-1915) correlated the prophylactic efficiency of plague vaccine with the virulence of the organism used for vaccine preparation

Although the main consensus of opinion, amply borne out by the experience of many workers, is that the smooth strains of organisms always make better prophylactic vaccines, yet the rough strains in certain cases have been found to possess some protective efficiency against infection by smooth strains of the same organism

The nature of the transition from the smooth to the rough phase has not yet been clearly established though various theories have been propounded claiming to explain this phenomenon It is quite possible that this change differs culturally, morphologically, serologically and immunologically in any particular organisms

Dr Cowan (1923), working with dissociation variants of *Streptococci* observed that some of the avirulent strains definitely protected against the virulent ones

Headley (1919) working on *B. aisepticus* derived a rough strain which markedly afforded protection against the smooth strain This particular rough strain differed from his other rough strains in not fermenting saccharose

Anderson (1929) and others have found that some protection was afforded by a rough vaccine of *B. aisepticus* in pigeons against a lethal dose of a smooth strain but their rough strain was reacting indifferently towards saccharose, sometimes fermenting it and at others not

White (1929) working on intestinal organisms claimed that the essential change in microbic-dissociation from smooth to rough implies the loss of non-protein carbohydrate—containing soluble specific component Although the intricate structures of various organisms generally infecting the human intestinal tract have been worked out during recent years yet we are not in a position to say definitely that the main antigens thus discovered are the only factors concerned in the protection of the individual against a particular infection It has been shown that there are a number of other constituents also For instance, in the organism complex of *B. typhosus*, besides the antigens we have exotoxins and endotoxins, etc

Whatever may be the difference in structure between the variants of a particular pathogenic organism, the main fact, of paramount importance to those interested in preventive medicine, is to ascertain which vaccine will give the best protection to an individual against a particular disease With this point in view we carried out certain experiments to investigate, in a simple way, the immunological relationship of the rough and the smooth variants of *B. typhosus* and to find out whether one variant can replace the other for protective purposes, and also as to which is the better of the two As a point of interest we also endeavoured to determine the efficiency of a mixed vaccine containing both the variants, this being the nearest approach to the whole organism In so far as the protective antigens were concerned we endeavoured to determine which vaccine is most competent to protect an individual against both types of infection Previous research has shown that the rough variant although usually associated with avirulence is not always completely so Thus in our experiments, the guinea-pigs receiving live rough cultures showed a definite mortality although the dose required to kill the animals was considerably higher than that of smooth type We tested the variants by their usual characters and proceeded to determine their minimal lethal doses for guinea-pigs (500 grms.) with a view to putting up controls and regulating the test doses

Experiment 1

Determination of the minimal lethal doses of rough and smooth 24 hours broth cultures of *B. typhosus* given intraperitoneally to guinea-pigs of 500 grammes body-weight

TABLE I

Cultures	Doses			
Rough	2 cc	3 cc	4 cc	5 cc
Number of guinea-pigs	2	2	2	2
Result	2 L	2 L	2 D	2 D

TABLE II

Cultures	Doses			
Smooth	1 cc	1·5 cc	2 cc	3 cc
Number of guinea-pigs	2	2	2	2
Result	2 L	2 D	2 D	2 D

L = Survived after 96 hours
D = Death within 96 hours

All the guinea-pigs used in the test (including controls) weighed 500 grammes each They were all kept under the same conditions and fed on the same scale of food

Three vaccines were prepared —

1 Smooth Vaccine (S V) from the smooth variant of *B typhosus*

2 Rough Vaccine (R V) from the rough variant of *B typhosus*

3 Mixed Vaccine (M V) containing both rough and smooth variant in equal parts

All the vaccines were prepared from 24 hours agar slopes, standardized by Brown's opacity tubes, the organisms killed by heat at 56°C for one hour and formalized (0·20 per cent)

The guinea-pigs were injected subcutaneously in the region of the groin with 1 cc of a vaccine containing 500 million organisms to the cc and a similar dose containing the same number of organisms was repeated on the 7th day On the 12th day following the second immunizing injection the guinea-pigs received the test dose, which consisted of a 24 hours broth culture of *B typhosus* intraperitoneally some receiving the rough and others the smooth variant (*vide* Table)

Controls were put up in each part of these experiments

Having ascertained that both the smooth and mixed vaccines protected better against each variant than the rough vaccine alone, we put up a greater number of guinea-pigs to compare the relative efficiency of the smooth and mixed vaccines

The results are shown in Tables III and IV

TABLE III

Immunization of Guinea-pigs by two subcutaneous doses of 500 millions each of Smooth (S V) and Mixed (M V) Vaccines at 7 days' interval Test doses of live broth cultures (24 hours) 12 days after the second immunizing doses intraperitoneally in each case Readings taken after 96 hours

	Guinea-pigs immunized by Smooth Vaccine			Guinea-pigs immunized by Mixed Vaccine			Control guinea-pigs		
1 Doses of live culture	1·5 c.c.	3 c.c.	4 c.c.	1·5 c.c.	3 c.c.	1 c.c.	1·5 c.c.	3 c.c.	1 c.c.
Guinea-pigs immunized by—	S V	S V	S V	M V	M V	M V	Control	Control	Control
Type of live culture	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Number of guinea-pigs	13	13	13	13	13	13	8	8	8
Result—									
Survived	11	12	8	11	12	11	3	0	0
Died	2	1	5	2	1	2	5	8	8
2 Doses of live culture	3 c.c.	1 c.c.	5 c.c.	3 c.c.	1 c.c.	5 c.c.	3 c.c.	4 c.c.	5 c.c.
Guinea-pigs immunized by—	S V	S V	S V	M V	M V	M V	Control	Control	Control
Type of live culture	Rough	Rough	Rough	Rough	Rough	Rough	Rough	Rough	Rough
Number of guinea-pigs	13	13	13	13	13	13	8	8	8
Result—									
Survived	10	8	1	11	9	5	3	1	0
Died ..	3	5	12	2	4	8	5	7	8

TABLE IV

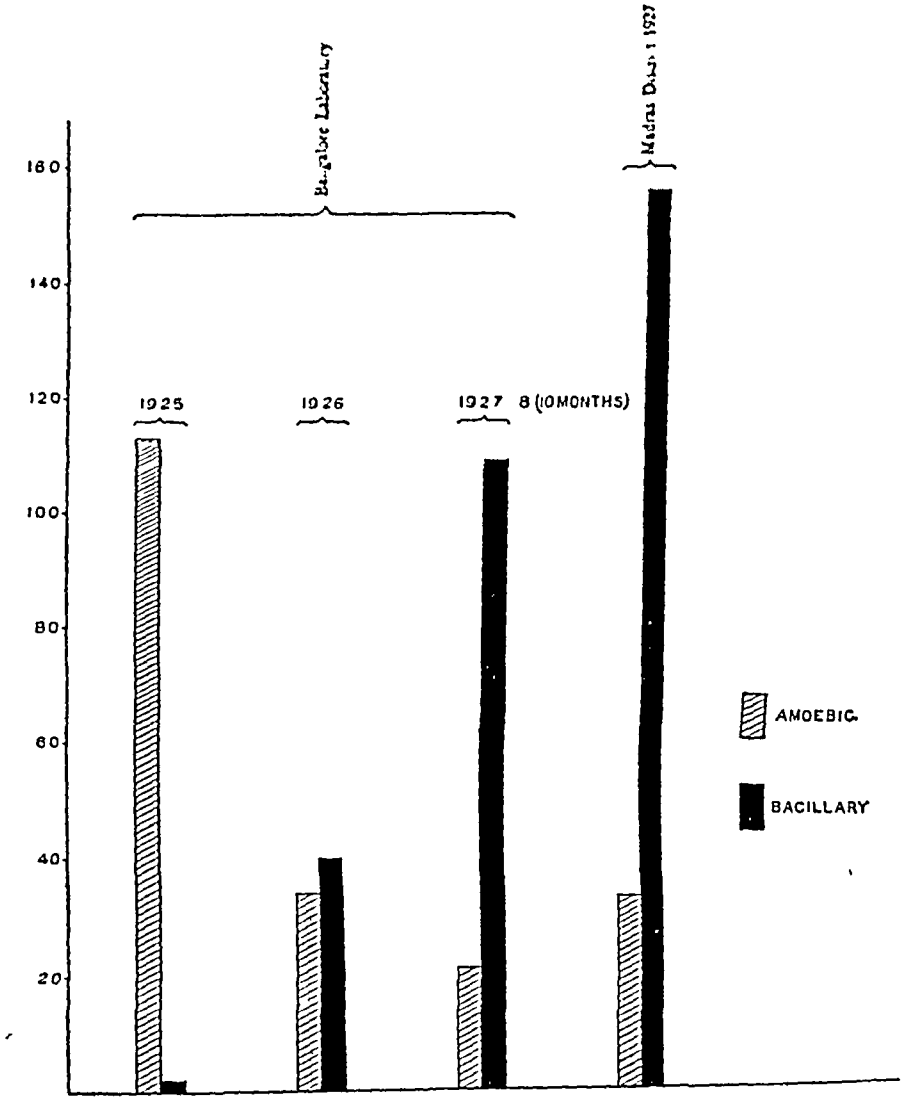
Immunization of Guinea-pigs by two subcutaneous doses of 500 million each of Rough (R V) Vaccine at 7 days' interval Test doses of live broth cultures (24 hours) 12 days after the second immunizing doses intraperitoneally Readings taken at the end of 96 hours

Control guinea-pigs for Smooth test doses	Doses of live culture	1·5 cc	3 cc	4 cc	Smooth test doses	1·5 cc	3 cc	4 cc
	Guinea-pigs	Control	Control	Control		Immunized by R V	Immunized by R V	Immunized by R V
	Number of guinea-pigs	2	2	2		6	6	6
	Type of live culture	Smooth	Smooth	Smooth		Smooth	Smooth	Smooth
	Result—							
	Survived	2	0	0		6	0	0
	Died	0	2	2		0	6	6
Control guinea-pigs for Rough test doses	Doses of live culture	3 cc	4 cc	5 cc	Rough test doses	3 cc	4 cc	5 cc
	Guinea-pigs	Control	Control	Control		Immunized by R V	Immunized by R V	Immunized by R V
	Number of guinea-pigs	2	2	2		6	6	6
	Type of live culture	Rough	Rough	Rough		Rough	Rough	Rough
	Result—							
	Survived	2	0	0		6	1	0
	Died	0	2	2		0	5	6

the Garrison of the district had shrunk to its present post-war dimensions—that is for the last six or seven years—the average number of cases of the group 'diarrhœa, dysentery and colitis' had kept surprisingly level at somewhere in the neighbourhood of one hundred per annum Sometimes diarrhœa would exceed dysentery, and vice versa, but the total would remain steady

With regard to dysentery, he pointed out that protozoal dysentery was shown in the records always in excess—very large excess—of bacillary

This district, it will be seen, was, until the end of 1925, a stronghold of amœbic dysentery, and appears to have been so from time immemorial The first columns of the Chart show the situation very clearly In justification of



this, Sir Leonard Rogers (1921) has been quoted to us, and it seems as though that authoritative writer does actually give the impression that protozoal dysentery is the chief dysentery in the Madras Presidency, an area practically coincident with the Madras Military district

Abroad, most countries have long recognized the high prevalence of bacillary dysentery, and in India, Acton and Knowles (1924) working amongst sections of the civil community have freely expressed their views as to the preponderance of bacillary dysentery over amœbic Cunningham (1918) also has found latent bacillary dysentery to be common in India Manifold (1928), working in Poona amongst soldiers, endorsed the view of Acton and Knowles and entirely reversed the dysentery figures for that district We ourselves, from personal experience, were aware of the striking preponderance of bacillary dysentery amongst troops in Mesopotamia, and set out therefore on our inquiry fully convinced that we should have the same experience in the Madras district

The present notes are on figures extracted from the results of an inquiry into the Intestinal Disorders of the Madras District, the period covered by the inquiry is from 20th April, 1927 to 31st January, 1928—about 10 months—during which time we were working in Bangalore

The dysentery figures for the Bangalore Laboratory for the years 1925, 1926 and 1927, together with the figures for both laboratories for 1927, are shown at the Chart

The year 1925 was the last under the old regime Its figures were —

Amœbic dysentery	113
Bacillary dysentery	2
Total	115

This shows the striking preponderance of amœbic dysentery over bacillary already mentioned, in fact the Madras district was one with 'an amœbic reputation'

The year 1926 was one of transition, during which one worker trained in modern methods and one of the old regime were employed The figures already show an improvement as is seen below —

Amœbic dysentery	34
Bacillary dysentery	36
Total	70

In the year 1927, our laboratory moved from Wellington to Bangalore where we produced the following figures —

Amœbic dysentery	21
Bacillary dysentery	109
Total	130

In the case of specimens from out-stations or those collected at night after eight o'clock, the routine was somewhat different A portion of the stool was placed by the sender in Teagues' glycerine and saline medium for examination for dysentery group organisms For examination for *E histolytica*, a cover glass preparation was made, fixed in Schaudinns' solution and transmitted in 75 per cent alcohol These smears, stained with Heidenhain's hæmatoxylin, were searched for amœbæ

We had misgivings at first about the preservative properties of glycerine and saline for the dysentery group bacilli, fearing that they might be outgrown in the heat during transit, but in actual practice we found that specimens collected in Madras in the hot weather in the morning, posted at night and reaching the Bangalore Laboratory about 25 to 28 hours later, contained viable organisms Experimentally inoculated faeces underwent a more severe test Normal faeces inoculated in the morning during the hot weather and remaining in the laboratory all day, were posted at night and travelled about 400 miles to Wellington Here vigorous colonies of the Flexner group bacilli were isolated from sowings made forty-eight hours after the specimens had been prepared

In the case of these out-station specimens an advance report was wired after preliminary microscopic examination, plates having been spread as in the case of local specimens

From the plates after twenty-four hours' incubation likely blue colonies were selected, three or four in each case These were inoculated into mannite, glucose, peptone-salt solution and broth If only glucose, or both glucose and mannite were fermented without gas production, the remaining sugars, namely, lactose and dulcite, were inoculated, the peptone-salt culture tested for indol by Bohme's method, the broth culture examined for motility and an agar slope sown This method was adopted mainly on account of the cost of dulcite

The broth cultures were killed in twenty-four hours with 0 3 per cent formalin and reserved for agglutination by Dreyer's method, which was used throughout High titre sera were prepared in the laboratory using well known strains of dysentery organisms, supplied by Lieut-Colonel H Marrian Perry, from the R A M College or from the National Collection at the Lister Institute The period of incubation for agglutination tests used throughout was the recognized four and a half hours at 56°C Towards the close of the inquiry we formed the opinion that this period of incubation was too short, but time would not permit of experiments directed towards discovering a more suitable period

Bacillary dysentery

As encountered by us this disease is not the virulent, fulminating and fatal affection that many textbooks would have one expect The vast majority of cases met with by us and included in the present report were extremely mild

—so mild in fact that the probability is great that only a small proportion of cases of the dysentery group of infection ever seek medical aid and thus come under observation by a laboratory

One of the most severe cases was one of Flexner infection one of the mildest Shiga dysentery

The very mildness of the disease explains the fact that the number of isolations of the causal organisms in our series is low It appears that it is only in cases where pain, fever, and frequency of stools persist that the individual, alarmed thereby, reports sick, perhaps many days after the onset of symptoms This is especially true in the case of the Indian soldier In such cases the possibility of isolating the offending organisms is remote

Of the cases diagnosed by cytological methods without isolation of the causal organism, the majority were those from whom the specimens were received too late in the disease, a proportion were cases in which specimens were sent in stale, with the causal organism dead or outgrown, and a number were sent in from a distance in the wrong preservative medium

In acute cases we found that a positive laboratory result could be obtained with certainty provided the specimen was despatched promptly by the hospital and reached the laboratory still warm In late cases this was not so In both classes of case, could we but have examined every stool passed, isolations would have been much more frequent, but unfortunately there was a tendency for the ward *to await the result from one stool before despatching another*

We are unable to say at the moment which strain of *B dysenteriæ* (*flexner*) was the most usual, partly because many of the strains actually isolated failed to agglutinate, on first being isolated, with V, W, X, Y, or Z high titre serum, and partly because so many thousands of agglutination tests were being carried out at the time that the final typing of these had to be left In most cases we had to be satisfied with agglutination by a polyvalent V-W-X-Y-Z serum Absorption tests were ruled out, at first by the absence of a suitable centrifuge, and later by lack of time and the very large amount of routine work—one day produced seventy-seven fæcal specimens, apart from anything in the way of urgent blood typing, throat swabs and so forth

The agglutinable strains we found to be agglutinable with polyvalent serum only after a variable number of sub-culturings, but in addition to these we collected from various sources some twenty-two further strains which were not yet agglutinable at the time the inquiry had to close owing to the transfer of one of us overseas

We are inclined to think that some of our strains are new, but are unable to make any definite statement on the subject, which should form the object of a fresh inquiry

B sonne we never encountered as a primary cause of dysentery Once only it was isolated in conjunction with *B flexner* and then not early in the

these cases too were dysenteries of some standing we were bound to suppose that at least in the three cases in which serum tests were applied, bacillary dysentery was the original primary cause of disease, and that the protozoal dysentery which we saw, was grafted on at a later stage, during which the individuals concerned happened to report sick and thus come under observation

TABLE III

	CASES SHOWING *E. HISTOLYTICA* (vegetative forms)							CASES SHOWING CHARCOT-LEYDEN CRYSTALS						
	DAY OF OBSERVATION						Total	DAY OF OBSERVATION						Total
	1st	2nd	3rd	4th	5th	6th		1st	2nd	3rd	4th	5th	6th	
No exudate	2		1	1			4	3						3
Bacillary *	1	2	1	1			5							
Amœbic †	1	1			1		6	2				1		3
TOTALS	7	3	2	2	1		15	5				1		6

*2 cases showed a rising agglutination curve against the Flexner group
†3 cases showed a rising agglutination curve against the Flexner group

THE CARRIER

(i) Bacillary dysentery

With bacillary dysentery outnumbering amœbic by about five to one, the healthy carrier might be expected to be found the common reservoir of virus Quite confident that we should find this to be the case we embarked on an investigation of the fæces of those individuals from whom the soldier might naturally be expected to contract dysentery, namely, the food-handling menial servant From long established custom these people have become indispensable, and their name is legion Cooks, langris, bhistis, bakers and the one thousand and one minions of the cook house and dining room and of the 'authorized' restaurants of Bangalore and of the other Garrison towns In addition to these were those special individuals examined as a routine in association with each case of dysentery

Three fæcal specimens were examined before each individual was employed, and three more at six-monthly intervals One thousand five hundred and forty-six individuals were so examined—some four thousand stools

Six, or 0 39 per cent, showed evidence of infection by one of the dysentery group of bacilli

Two of these were cases of frank, acute, bacillary dysentery, the remainder were carriers, but none was in any way associated with any case of dysentery which came to light

We conclude from the above that the healthy carrier of the dysentery group of organisms is comparatively rare, and consider that, since bacillary dysentery is encountered by us is so extraordinarily mild, the reservoir of virus is the ambulent mild case, which never comes under medical observation This could not be checked, bacteriologically, in the time, from the excreta in public latrines, because the laboratory was already too fully occupied, but a latrine group visited daily during April, May and June 1927 frequently showed the presence of suspicious mucoid stools, sometimes with macroscopic blood

(*ii*) *Amœbic dysentery*

With only 16 15 per cent of our cases found to be amœbic we should have expected a correspondingly low carrier rate of *B histolytica*

Conjointly with the bacteriological examination of the foregoing individuals, the same four thousand old specimens were examined microscopically for evidence of *E histolytica* infection

Three hundred and thirty-nine, or 21 93 per cent, showed *E histolytica* cysts

Direct examinations of the fæces were made in double strength iodine solution Specimens were unfortunately not ideal for the examination for cysts, being mainly from the soft stool following a saline purge Had we been able to inspect the whole specimen ourselves and examine the mucous coat of a solid stool, we are certain the numbers of carriers detected would have been much higher Workers in Great Britain have reported a carrier rate of ten per cent or more in permanent residents of that country (Dobell and O'Connor, 1921) hence we think our figure of 21 93 per cent much lower than might have been expected

The carrier rate of 21 93 per cent seems to us to be a fairly accurate estimate of the cyst content of the unsuitable material examined The average weekly figure was from eighteen to twenty per cent of positive findings, by ordinary direct methods During two months an intensive search was made by concentrating the cysts in all specimens and comparing the findings with those obtained by direct examination By so doing the findings were increased in one week to forty-two per cent, but this high figure was not maintained, and in an average week the positives obtained by concentrating were from twenty to twenty-five per cent, an increase of two or three per cent, only, over the positive findings by the direct method

Table IV gives the findings in our search for carriers of the dysentery group of bacilli and of *E histolytica*

Table V gives the numbers of cases of dysentery contrasted with the percentage of food-handling servants found to be carriers

TABLE IV

Total cases	D Group	Percentage	E Group	Percentage	*E histolytica*	Percentage	*I coli*	Percentage	Flagellates	Percentage	Helminths	Percentage
1516	6*	0·39	1	0·26	339	21·93	332	21·18	88	5·69	237	15·33

*Two cases of acute bacillary dysentery

TABLE V

	Number of cases	Carrier rate, per cent
Bacillary dysentery	109	0·39
Amœbic dysentery	21	21·93

SEROLOGICAL EXAMINATION OF THE SERA OF PATIENTS

Realizing that many of the dysentery cases were very mild, and might not come under observation until too late for the causal organism to be isolated, and that many specimens would be received stale, we arranged to investigate the agglutinin response in the sera of such cases

A series of six tests per case were aimed at, and orders were issued for blood to be withdrawn at four-day intervals, beginning with the first day of observation Further withdrawals were made on the fourth, eighth, twelfth, sixteenth and twentieth days

Oxford Standard Cultures were used and each serum was put up against *B flexneri*,—V, W, X, Y and Z strains—and against *B shiga* Dreyer's method of agglutination was used and the tubes were read after four and a half hours' incubation, and again after standing overnight As mentioned already, we concluded this period was too short an incubation for strains of dysentery bacilli freshly isolated, and in the case of Oxford Cultures with patients' sera this also seemed to be true, for occasionally, where racks were left in the water bath by a mistake from early morning until late at night, when the laboratory finally closed readings had moved on very appreciably, two, three or even more tubes Readings from such accidentally extended incubations have been excluded from this report

In actual practice it was found impossible to investigate all cases serologically, for in some cases blood withdrawals were not indicated In seventeen out of the seventy-one cases in which sera was obtained, only one specimen per case was received Of these seventeen cases, all agglutinated some or all of the Flexner strains but not Shiga It was subsequently discovered that in one of these cases anti-dysenteric serum had been used

Forty-one cases, including nine who had received anti-dysenteric serum, showed a steadily rising curve of agglutinin response to some or all of the Flexner strains Shiga was not agglutinated

Thirteen cases showed no rise whilst under observation, and were lost sight of after twenty days These cases included four which had had anti-dysenteric serum

Though these numbers are small, they are definite indication that the common cause of bacillary dysentery in this district is one of the Flexner group

TABLE VI

	Anti-dysenteric serum administered	Anti-dysenteric serum not administered
Rising agglutinin response	9	38
Agglutinins present	1	16
No agglutinins present	4	9

THE HEALTHY BRITISH SOLDIER AS A CARRIER

With so high a rate of *E histolytica* carriers amongst food-handling menials in Barracks, we were interested to know whether these people were a danger to the soldier

It seemed useless to investigate the carrier rate amongst Indian soldiers who might be expected to show a rate comparable with that of the food-handlers, hence we examined the first available hundred British soldiers whom we could discover to be suitable subjects

All who showed documentary evidence of any intestinal disorder, as evidenced by medical history sheets, were rejected as unsuitable Such men as showed no evidence of previous dysentery or diarrhœa, and who, after careful questioning showed no suggestion of having at any time suffered from any disorder which might conceivably have been dysentery, were selected, including as many as possible of recent arrivals (i e, with less than one year in India)

With some difficulty we secured one hundred suitable subjects and examined their fæces The result is shown at Table VII

Ten per cent might reasonably have been expected to have been harbouring *E histolytica*, but we did not expect to find twenty-three per cent carriers, especially as amœbic dysentery has been shown to be of almost negligible prevalence

The cysts were all measured and found to be 8 05 micron This is the same small cyst seen in the stools of the Indian food-handlers and on account of the low incidence of amœbic dysentery, we are inclined to think it must be

a harmless parasite and not the cyst of the causal entamœba of protozoal dysentery

At the time when the twenty-one cases included as amœbic dysentery were being investigated, it did not occur to us to measure the cysts found during convalescence This is unfortunate, as such measurements could most profitably have been compared with those made in the case of carriers

TABLE VII

Method of examination	Number of cases	Findings			
		E. histolytica	*E. coli*	*Giardia*	Helminth.
Direct (microscopical)	100	18	21	11	10
Concentration	100	23	23	16	1

THE NATURE OF AMŒBIC DYSENTERY

As an outcome of our enquiry, we are left more than ever in the dark as to the real nature of amœbic dysentery and the significance of the presence of cysts in the fæces

Is the *Entamœba histolytica* ever the cause of primary dysentery?

Surely the main object in life of this protozoon is to feed and grow quietly, in the sub-mucosa of the large intestine, and from that point of vantage, steadily to reproduce its species by the production of cysts The last thing it wishes to do is to be swept out of the intestine unencysted, in the vegetative and vulnerable state, to perish almost immediately when exposed to the atmosphere It seems unreasonable that *E histolytica* should voluntarily produce dysentery in its host

It is a known fact that many who harbour *E histolytica* pass through life without ever developing any kind of dysentery

What is more reasonable to suppose than that amœbic dysentery is always a secondary disorder, and that it appears only when a victim of intestinal amœbiasis contracts some bacillary infection of the large intestine? What again is more reasonable to suppose than that the most suitable bacterial infection to produce the proper conditions for *E histolytica* to appear in its vegetative stage in the stools is an attack, however mild, of bacillary dysentery, with its colliquative necrosis of the mucosa of the large intestine and the consequent opening of the sites of amœbic infestation?

The bacillary infection passes away, perhaps hardly noticed, as a mild diarrhœa, to leave open infected ulcers, extruding vegetative amœbæ, and thus is developed the syndrome known as amœbic dysentery

If this is actually the case, we believe that the small 8 05μ cyst generally found by us is not pathogenic and thus the infrequency of amœbic dysentery in the district is explained

SUMMARY

(1) Protozoal (amœbic) dysentery has long been regarded as the chief dysentery in the Madras district

(2) In actual fact bacillary outnumbers amœbic dysentery by about five to one at least

(3) The commonest causal organism is a bacillus of Flexner type

(4) That bacillary dysentery as seen by us is mild, but present all the year round

(5) That healthy carriers of the dysentery group of organisms are rare 0 34 per cent

(6) Indian carriers of *E histolytica* are common At least 21 93 per cent

(7) The British soldier carries *E histolytica* at approximately the same rate

(8) It is conceivable that the *E histolytica* of this district is non-pathogenic

(9) It is equally conceivable that amœbic dysentery is always secondary to bacillary

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THE FEMALE OF *PHLEBOTOMUS NICNIC* BANKS, 1919

BY

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[Received for publication, February 15, 1930]

Phlebotomus nicnic was described by Banks (1919) from specimens collected at Los Baños, Philippine Islands in July 1915 His original description shows that the species belongs to the '*minutus*' group of this genus and it is interesting that the insect is recorded as biting man, a habit which is not usual in the members of this group Banks (1919*a*) states that 'its bite is extremely severe, even more painful than that of most mosquitoes, and the wheal remains itchy for a day or more'

Sinton (1928), in his attempt to clear up the very confused synonymy of of the Asiatic species of *Phlebotomus*, pointed out that considerable doubt existed as to the relationship of this species to *P minutus* Rondani, *P perturbans* de Meijere and *P babu* Annandale The fact that *P nicnic* is a vicious biter of man made it unlikely that it was identical with *P minutus* or *P babu*, but its possible identity with *P perturbans* from the neighbouring island of Java could not be neglected It was suggested that when opportunity arose, the species should be redescribed with special reference to the morphology of the buccal cavity, pharynx, spermathecæ, etc, on which characters more recent research has based the identification of the species of this genus Thanks to the kindness of Dr C Manalang of the Philippine Health Service, I have obtained one of the paratype females of this species, and the description given below was made from an examination of this specimen

SUMMARY OF ORIGINAL DESCRIPTION

The salient diagnostic characters in the original description of *P nicnic* given by Banks (1919) may be summarized as follows —

'*Male and female*—Greyish ochraceous to brownish buff, with slight silvery reflections at the ends of some of the squamous hairs which so abundantly cover the body Seen by transmitted light head and thorax are honey yellow, abdomen and legs buff' 'Proboscis one-half length of entire

head Palpi* with first and second segments subequal, the former curved basad, third slightly longer and thinner, fourth and fifth subequal and nearly filiform, the latter bulbous basad Antennæ with first segment cythform, second spherical, third three times length of fourth' 'Abdomen—with semi erect hairs as long as the segments and evenly scattered over the tergites and sternites' Wings—twice length of abdomen, their greatest width one-third their length in both sexes Petiole of second longitudinal equals anterior branch of fork' Genitalia—hypopygium of male twice length of last abdominal segment, ventral styles fleshy, straight or slightly curved, setose along sides and at rounded apex Harpes asymmetrically spatulate and with four stout, curved spines at apex Penis slender, constricted before apex which is obconical' Body length Male 1 673 mms , female 2 167 mms Wing length Male 1 44 mms female 1 71 mms Luzon, Laguna, Los Baños (Charles S Banks)'

'Type—Male and female No 18192 in entomological collection, College of Agriculture, Los Baños, P I Several additional specimens collected at the same time and place are labelled as paratypes'

Fresh description of *Phlebotomus mcmic* Banks, 1919 (♀)

The pinned specimen received from Dr Manalang was labelled 'Los Baños, P I, 28-vii-1915 (C S Banks)' and is apparently one of the paratypes mentioned above The specimen was examined in the dry state and then passed through caustic potash solution, stained and mounted in balsam

Appearance in the dry state

The insect was of medium size, greyish brown in colour The integument of the dorsum of the thorax was very dark brown and that of the rest of the body yellowish brown The hairs on the thorax were golden brown and the pleuræ showed no scales The abdominal hairs were also golden brown and those on the dorsum were recumbent except on the first segment

The wings had a bluish-golden iridescence and were covered with yellowish grey hairs, which appeared infuscated along the margins of the wing, especially anteriorly There were a few scales at the wing bases The legs looked dark grey in some lights, while in others they showed silvery reflections with a yellowish tinge The antennæ and palps were yellowish

As the specimen was 15 years old, it is possible that changes in coloration have occurred from that seen in the fresh state, more especially in the colour of the integument

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* Palpal segment one of Banks' description corresponds to segments one and two of the present description, segment two is segment three, segment three is segment four and segments four and five are segment five

Appearances in stained and mounted specimens

The measurements of the different parts of the body are given in the attached Table

The total length of the insect is 2 33 mm , of which the abdomen forms half The cicatrices on the dorsum of the abdomen showed that the insect belongs to the 'recumbent-haired' group

The *buccal cavity* (Plate XV, fig 7) shows a small oval pigmented area and the armature is not well developed The *pharynx* (Plate XV, fig 6) is not markedly dilated distally, its length is almost three times its greatest breadth The pharyngeal armature shows proximally 10 to 12 long fine teeth, and more distally a series of transverse ridges edged with minute short teeth or serrations

The *palps* (Plate XV, fig 2) have a formula of 1, 2, 3, 4, 5 The 3rd segment is slightly shorter than the 4th, while the 5th is very long, being twice the length of the latter As noted by Banks (1919), the combined lengths of segments 1 and 2 equal that of 3 Newstead's spines form a compact mass near the base of the middle third of segment 3 and number about 10 to 14

The *antennæ* (Plate XV, figs 3, 4 and 5) have paired geniculate spines on all segments from III to XV inclusive These are comparatively short and stout and rather asymmetrically placed Segment III is relatively short, being only about half the length of segments XII to XVI, but it is slightly longer than the combined lengths of segments IV and V

The *wing* (Plate XV, fig 1) measures 1 74 mm in length and is about 3½ times as long as broad The proximal fork of the 3rd vein is distinctly nearer the base of the wing than the fork of the 4th a is equal to β, while δ is relatively large

The *female genitalia* —The spermatheca (Plate XV, fig 9) is smooth and sausage-shaped with a narrow stem, it is nearly three times as long as broad The furca (Plate XV, fig 10) is like that found in other members of the '*minutus*' group The post-genital plate (Plate XV, fig 8) carries two spines, which unfortunately are broken in this specimen

Differential diagnosis

The absence of erect hairs on the dorsum of the abdomen and the smooth outline of the spermatheca at once distinguishes this insect from any of the erect-haired species

The morphology and the very poor development of the buccal and pharyngeal armatures differentiate the species from the other members of the 'recumbent-haired' division (*Cf* the figures given by Sinton, 1927, Patton and Hindle, 1928, and Adler and Theodor, 1927, 1929)

Differential diagnosis of the male

Banks (1919) states that the distal segment of the superior clasper bears four stout curved apical spines and figures these

TABLE

Phlebotomus niene *Banks* 1919 (♀) *

	Structures	Lengths in mms	Relative lengths, ratios, etc
Body	Clypeus and head	0·313	
	Thorax	0·670	
	Abdomen proper	1·113	
	Superior clasper	0·170	
	Total length	2·33	= 1·31 × wing length, = 0·91 × hind leg
	Labium	0·228	
	Epipharynx	0·220	$\frac{P}{E} = 2·95$ $\frac{P}{L} = 2·81$
	Pharynx length	0·156	
	Pharynx breadth	0·051	
Antenna	Segment III	0·111	III > IV + V IV = V = VI
	Segment IV	0·066	IV + V + VI < XII to XVI
	Segment V	0·069	
	Segment VI	0·069	Antennal formula $\frac{2}{\text{III to XV}}$
	Segments XII to XVI	0·290	= 2 × IIIrd
	Total length	1·240	= 0·86 × IIIrd, = 0·43 × XII to XVIth
Palp	Segment 1	0·030	Palpal formula, 1, 2, 3, 4, 5
	Segment 2	0·090	Relative lengths, 2·2, 6·6, 9·1, 10, 20
	Segment 3	0·123	= 1st + 2nd
	Segment 4	0·135	
	Segment 5	0·270	= 2 × 5th
	Total length	0·648	= 7 × 2nd, > 5 × 3rd
Wing	Length	1·713	= 3·56 × breadth, = 0·67 × hind leg
	Breadth	0·485	$\frac{\alpha}{\beta} = 1·0$ $\frac{\beta}{\gamma} = 1·21$ $\frac{\alpha}{\gamma} = 1·21$ $\frac{\delta}{\alpha} = 0·52$
	α	0·328	
	β	0·328	
	γ	0·270	
	δ	0·170	$\frac{\alpha}{\epsilon} = 0·70$ $\frac{\beta}{\epsilon} = 0·70$ $\frac{\theta}{\epsilon} = 1·90$ $\frac{\alpha+\beta}{\theta} = 0·74$
	ϵ	0·470	
	θ	0·885	$\frac{\text{Wing length}}{\theta} = 1·95$
	π	0·071	
Hind leg	Femur	0·685	> ⅓ leg
	Tibia	0·895	> ¼ leg
	Tarsus, segment 1	0·400	
	Tarsus, segments 2 to 5	0·585	
	Total length	2·57	(Not including coxa and trochanter)
	Spermatheca, length	0·156	= 2·9 × breadth
	Spermatheca, breadth	0·054	

* Paratype female

The apical situation of these spines prevents confusion of this species with *P zeylanicus*, *P malabaricus*, etc (i e, Group 4 of Sinton 1928), but until a fuller description on the male is available its differential diagnosis from *P minutus*, *P babu*, etc (i e, Group 3 of Sinton, 1928) will be a matter of considerable difficulty

It is clear from the above descriptions that *Phlebotomus nicnic* Banks, 1919, is a distinct species and must therefore be included in the list of Asiatic sandflies

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Sinton, J A	(1927)	*Ind Jour Med Res*, XV, 1, pp 29—32
Idem	(1928)	*Ibid*, XVI, 2, pp 297—321

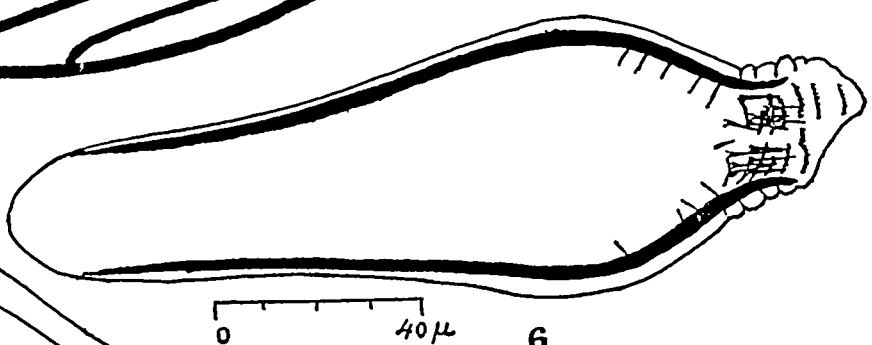
Explanation of Plate XV

Phlebotomus minic Banks, 1919 (♀)

Fig 1 Wing
„ 2 Palp N The spines of Newstead
„ 3 Antennal segments XV and XVI
„ 4 Antennal segments IX and X
„ 5 Antennal segments III, IV and V
„ 6 Pharynx
„ 7 Buccal cavity
„ 8 Post-genital plate
„ 9 Spermatheca
„ 10 Furca

PLATE XV

1



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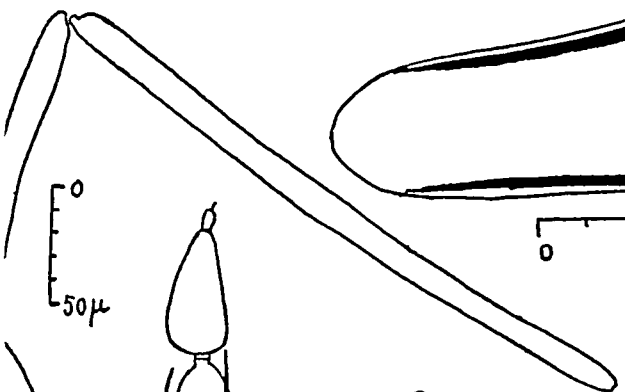

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4

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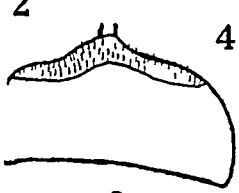

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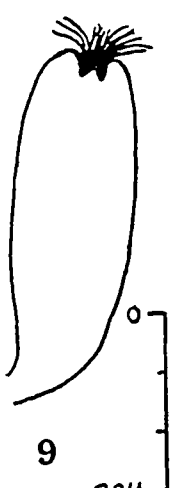

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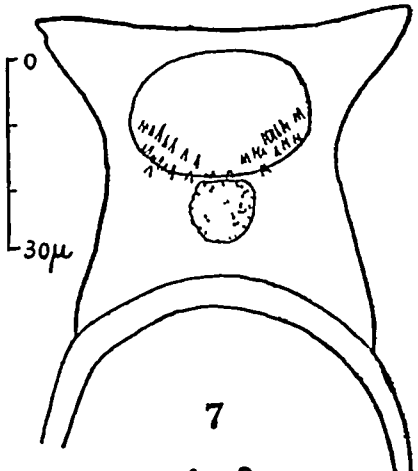

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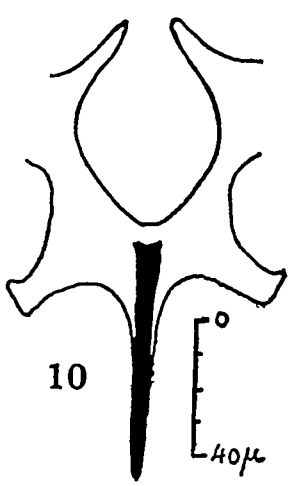

10

SOME NEW SPECIES AND RECORDS OF *PHLEBOTOMUS* FROM AFRICA

BY

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[Received for publication, March 19, 1930]

THROUGH the kindness of Colonel S R Christophers, C I E, F R S, Major W F M Loughnan, M C, R A M C, and Dr C B Symes, I have received a number of specimens of *Phlebotomus* from different parts of Africa, among which several new species have been found While studying these specimens it was found necessary to search literature for the recorded distribution of the different African species and as the data may prove helpful to other workers the results are given below

THE AFRICAN SPECIES OF THE GENUS *Phlebotomus*

The following species of *Phlebotomus* have been recorded from different parts of Africa* —

1 *P papatasi* (Scopoli), 1786
2 *P duboscqi* Neveu-Lemaire, 1906
3 *P major* var *perniciosus* Newstead, 1911
4 *P roubaudi* Newstead, 1913, 1914 (♂)
5 *P sergenti* Parrot, 1917 (♂), Franca, 1918 (♀)
6 *P minutus* Rondani, 1843
7 *P minutus* var *antennatus* Newstead, 1912 (♀), 1920 (♂)
8 *P africanus* Newstead, 1912
9 *P ingrami* Newstead, 1914 (♀)
10 *P bedfordi* Newstead, 1914 (♀)
11 *P similimus* Newstead, 1914
12 *P signatipennis* Newstead, 1920 (♀)
13 *P fallax* Parrot, 1921

*See foot-note to Summary

14 *P. parroti* Adler and Theodor, 1927

15 *P. squamipleuris* Newstead, 1912 (♀), Sinton, 1923 (♂).

Of these insects, numbers 1 to 5 appear to belong to the erect-haired species, while numbers 6 to 14 are probably all recumbent-haired species mainly of the *minutus* group. Unfortunately the females of the latter group are very difficult to identify, except by means of such characters as the morphology of the buccal and pharyngeal armatures, the spermathecæ, etc., and in numbers 9 to 13 these do not seem to have been studied in detail. Thanks to the work of Adler and Theodor (1926, 1927) we have very good descriptions of the diagnostic characters of *P. minutus*, *P. parroti* and *P. africanus*. In the case of *P. africanus* it seems very certain that several species have been included under this name in the past, so it is essential that the type specimens of this species should be re-examined to see if it is the same species as that so carefully described by Adler and Theodor (1926, 1927) from Palestine. One of the paratype females of this species from Southern Nigeria which was examined by me seemed identical with the Palestine species, but as the type of this species came from N. E. Rhodesia (Newstead, 1912) the identity of the two requires confirmation.

Recorded Distribution of *Phlebotomus* in Africa.

As far as can be ascertained the following are the principal records of the distribution of *Phlebotomus* in Africa and from these it would appear that there are many blanks in our knowledge of this subject.

(1) *Phlebotomus papatasii*

Algeria—(Nicolet, 1912, Foley and Leduc, 1912, Sergent, Ed., 1914, Newstead, 1914, Parrot, 1917, 1918, 1922, 1926, Parrot and Donatien, 1922, 1927, etc.) Tunisia—(Langeron, 1912, 1921, Newstead, 1914, Chatton and Blanc, 1918, Roubaud and Colas-Belcour, 1927, Weiss, 1927, Colas-Belcour, 1928) Morocco—(Delanoe, 1916, Vialatte and Parrot, 1921) French Sudan—(Roubaud, 1913, Suldey, 1926, 1927) Mauretania—(Picard, 1909) Spanish Guinea—(Pittaluga and de Buen, 1917) Egypt—(Newstead, 1912, Willcocks, 1917) Anglo-Egyptian Sudan—(Austen, 1909, Newstead, 1912, 1914, King, 1911, 1913, 1914, Archibald, 1923) Italian Somaliland—(Franchini, 1925).

(2) *Phlebotomus duboscqi*

French Sudan—(Neveu-Lemaire, 1906, Austen, 1909, Suldey, 1926) Mauretania—(Roubaud, 1913) Chad Territory—(Le Gac, 1928) Ashanti—(Austen, 1909) Southern Nigeria—(Austen, 1909, Simpson, 1914) Sierra Leone—(Dalziel and Johnson, 1915).

(3) *Phlebotomus major* var. *perniciosus*

Algeria—(Sergent, Ed., 1914, Parrot, 1917, 1918, 1922, 1926, Parrot and Donatien, 1922, Sergent, Ed. and Trollet, 1923, Sergent, Ed., etc., 1925, etc.)

Tunisia—(Langeron, 1912, Chatton and Blanc, 1918, Larrousse, 1923, Roubaud and Colas-Belcour, 1927, Weiss, 1927, Colas-Belcour, 1928) Morocco—(Vialatte and Parrot, 1921) Spanish Guinea—(Pittaluga and de Buen, 1917)

(4) *Phlebotomus roubaudi*

Mauretania—(Newstead 1913, 1914)

(5) *Phlebotomus sergenti*

Algeria—(Parrot, 1917, 1918, 1921*b*, 1922, 1926, Sergent, Ed., etc., 1925) Tunisia—(Langeron, 1921, Parrot, 1921*b*, Larrousse, 1923, Roubaud and Colas-Belcour, 1927) Morocco—(Vialatte and Parrot, 1921)

(6) *Phlebotomus minutus* *

Algeria—(Parrot, 1921*a*, 1926, Sergent, Ed., etc., 1925, Adler and Theodor, 1929) Tunisia—(Newstead, 1914, Langeron, 1921, Parrot, 1921*a*) French Sudan—(Suldey, 1926 1927) Sierra Leone—(Alcock, 1912) Anglo-Egyptian Sudan—(Archibald, 1923) Italian Somaliland—(Franchini, 1925)

(7) *Phlebotomus minutus* var. *antennatus*

Northern Ashanti—(Newstead, 1914) Gold Coast—(Newstead, 1912, 1920, Simpson, 1914)

(8) *Phlebotomus africanus* †

Algeria—(Sergent, Ed., 1914, Newstead, 1914, Parrot, 1917, 1918, 1922, Parrot and Donatien, 1927, Pons-Leychard, 1926, etc.) Tunisia—(Chatton and Blanc, 1918, Roubaud and Colas-Belcour, 1927) Morocco—(Vialatte and Parrot, 1921) Senegal—(Roubaud, 1913, Parrot, 1921*b*) Gold Coast—(Newstead, 1912, 1920, Simpson, 1914, Macfie, 1915, Carter, Ingram and Macfie, 1920) Ivory Coast—(Roubaud, 1913, Newstead, 1913, 1914) Chad Territory—(Le Gac, 1928) Northern Nigeria—(Newstead, 1912, Taylor 1929) Southern Nigeria—(Newstead, 1912, 1914) Northern Ashanti—(Newstead, 1914) West Africa—(Summers, 1913) Belgian Congo—(Tonnoir, quoted by Larrousse, 1928, Adler and Theodor, 1929) Anglo-Egyptian Sudan—(Newstead, 1912, 1914) Northern Rhodesia—(Newstead, 1912, Neave, 1912) Nyasaland—(Newstead, 1912, 1914, Neave, 1912) East Africa—(Summers, 1913) Portuguese East Africa—(Newstead, 1914) Tanganyika Territory—(Ann. Med. Rept. Tanganyika Territory, 1921) Transvaal—(Newstead, 1914, Parrot, 1921*b*) Mauritius—(Loughnan, 1929)

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* *See* footnote to Summary.

† In many instances it is doubtful whether the species recorded is *P. minutus* Rondani or *P. africanus* Newstead, which was formerly considered as a variety of *P. minutus*. The *Phlebotomus rondani* recorded by Clapier (1921) from French Equatorial Africa is possibly a misprint for *P. minutus* Rondani.

(9) *Phlebotomus ingrami*

Ivory Coast—(Newstead, 1920) Northern Ashanti—(Newstead, 1914) Uganda—(Larrousse, 1928)

(10) *Phlebotomus bedfordi*

Transvaal—(Newstead, 1914, Parrot, 1921*b*)

(11) *Phlebotomus similimus*

Northern Ashanti—(Newstead, 1914) Southern Nigeria—(Newstead, 1914)

(12) *Phlebotomus signatipennis*

Gold Coast—(Newstead, 1920)

(13) *Phlebotomus fallax*

Algeria—(Parrot, 1921, 1921*a*, 1926, Parrot and Donatien, 1927) Tunisia—(Parrot, 1921, Langeron, 1921)

(14) *Phlebotomus parroti*

Algeria—(Adler and Theodor, 1927, 1929) Tunisia—(Colas-Belcour, 1928)

(15) *Phlebotomus squamipleuris*

Anglo-Egyptian Sudan—(Newstead, 1912) Gold Coast—(?) (Macfie, 1915)

(16) *Phlebotomus* spp

Algeria—(Niclot, 1912, Vialatte, 1916, Piquemal, 1923, Bidault, 1923) Upper Volta—(Legendre, 1927) West and Central Africa—(Theobald, 1903, Austen, 1909) Southern Nigeria—(Clark, 1914) Gold Coast—(Newstead, 1920) Belgian Congo—(Newstead, Dutton and Todd, 1907) Egypt—(Pressat, 1905, Austen, 1909, Hartley, 1918) Anglo-Egyptian Sudan—(Balfour, 1906, 1911, King, 1911) Uganda—(Theobald, 1903) Tanganyika Territory—(Manteufel, 1912, Morstatt, 1921) Northern Rhodesia—(Neave, 1911)

Phlebotomus IN MAURITIUS

Through the kindness of Major W F M Loughnan, MC, RAMC, I have received about 50 specimens of *Phlebotomus* from Mauritius These on external examination resembled *P africanus* but in stained and mounted specimens they were found to be typical specimens of *P babu*, the common Indian species The only other record of *Phlebotomus* from this island is that of *P africanus* by Loughnan (1929) There seem to be no records of this genus from the neighbouring island of Madagascar, where Legendre (1918) reports them as absent from the town of Tamatave

Phlebotomus IN MOMBASA

There seem to be no definite records of this genus from the seaboard of East Africa, apart from those of *Phlebotomus* spp by Manteufel (1912) and

Morstatt (1921) from Dar-es-salaam. As noted above *P. africanus* is reported from East Africa and Aders (1913) says no *Phlebotomus* were obtained in the Zanzibar Protectorate while Ross (1913) makes a similar report for Kenya Colony.

Dr. C. B. Symes has kindly sent me a number of *Phlebotomus* caught in Mombasa during November 1929, and among these there have been found three species of the recumbent-haired group of which two are apparently new of science.

1. PHLEBOTOMUS SYMESI *sp. n.*

This new species is described from 13 females and 3 males collected at Mombasa, during November, 1929. I have great pleasure in dedicating this species to Dr. C. B. Symes, who collected them.

Phlebotomus symesi (♀).

Appearance in Dry State.

A medium-sized *Phlebotomus* with recumbent dorsal abdominal hairs. General appearance very dark grey. Integument very dark greyish-brown, except for the sides of the thorax which were yellowish-grey. Halteres black. No scales on pleuræ. Wings with golden iridescence, hairs greyish. Abdominal hairs golden yellow, sleekly arranged on dorsum and sides but slightly ruffled on venter. Legs, palps and antennæ varying from light to dark grey according to the light.

Appearance in Stained and Mounted Specimens.

The measurements of the type and three paratype females are given in Table I, in which the ratios, etc., have been calculated from eight specimens.

The *total length* of the insect is 2·40—2·54 mms. The abdomen proper is about twice the length of the thorax. The form of the cicatrics on the dorsum of the abdomen show that it is a recumbent-haired species.

The *buccal cavity* (Plate XVI, fig. 3) rather pear-shaped with the broad end posteriorly. The teeth are well developed and number 17—20. The lateral ones are wide, while the median ones are much narrower. The points of the teeth are difficult to see against the dark pigmented area and look conical under these conditions, but when dissected out are found to terminate in long thin points (Plate XVI, fig. 6). They are arranged in a curved line with the concavity posteriorly, while in some specimens they seem to extend so far laterally as almost to form a semi-lune.

The *pharynx* (Plate XVI, fig. 2) is stout in character and not so markedly dilated posteriorly as in some other species, its greatest width posteriorly is about twice that of the narrower anterior portion and its length is about 2½ times its greatest breadth. There is a conspicuous armature which gives the impression of imbricated scales with serrated ends pointing backwards. The *epipharynx* is long and fairly stout. The ratio palp over epipharynx is 3·4—3·6.

The *antennæ* (Plate XVI, figs 4 and 5) have paired geniculate spines on all segments from III to XV inclusive these are of moderate length, those on the terminal segments almost reaching the succeeding articulation Segment III is almost twice the length of segment IV and almost half that of segments XII to XVI The end of segment III does not reach as far as the end of the proboscis The terminal segment of the antenna is markedly elongated

The *palps* (Plate XVI, fig 10) have a formula of 1, 2, (3, 4) 5, and the relative lengths of the segments, as taken in 8 specimens, is 2 9, 7 1, 10, 10, 21 5 Very constant features seem to be that the combined lengths of segment 1 and 2 equal that of segment 3, which is also equal to segment 4 Segment 5 is usually slightly greater than the combined lengths of segments 3 and 4 Newstead's spines are situated on the basal third of segment 3 and number 18—20

The *wing* (Plate XVI, fig 1) is broadly lanceolate and about 3 3—3 5 times as long as broad α is about ½—⅔ the length of β and the ratio γ over α is about 0 3—0 5 The proximal fork of the 2nd vein is distinctly proximal to the fork of the 4th

The *hind leg* is longer than the body The femur forms about one-fourth of the leg and the 1st tarsal segment about one-sixth

The *spermatheca* (Plate XVI, fig 7) is oval, gradually merging into a very wide duct (Type A of Adler and Theodor, 1927) Its length is twice its breadth in well displayed specimens and it sometimes shows a few faint transverse striations on its surface The *post-genital plate* (Plate XVI, fig 9) usually carries two spines but some specimens show three The *furca* (Plate XVI, fig 8) is of the usual type

Phlebotomus symesi (♂)

The appearance in dry specimens was very similar to that of the females

Appearance in Stained and Mounted Specimens

The measurements of the type and two paratype males is given in Table II

The *total length* of the insect is about 2 45—2 6 mms

The *buccal cavity* (Plate XVII, fig 8) has a small but distinct pigmented area, oval in shape The teeth are large and number about 12—14, laterally they are inclined to be replaced by irregular toothed ridges The *pharynx* (Plate XVII, fig 8) resembles that in the female but is more slender The armature in both sexes is very similar but less developed in the male The *epipharynx* is slender and the ratio palp over epipharynx about 3 74—3 90

The *antennæ* (Plate XVII, figs 6 and 7) have single geniculate spines on all segments from III to XII inclusive, but unfortunately in none of the specimens were any segments present beyond the latter one Segment III is shorter than the combined lengths of segments IV and V The tip of the proboscis is almost level with the distal end of segment IV

The *palps* (Plate XVII, fig 2) have a formula of 1, 2, (3, 4), 5, and the relative lengths of the segments of the three specimens is 3 7 10 10 21 4

Newstead's spines are situated on the basal third of segment 3 and number about 4—6

The *hind leg* is almost equal in length to the body

The *wing* (Plate XVII, fig. 1) is relatively narrower and more pointed than in the female, being almost 4 times as long as broad. The ratios of the various parts is more or less similar to those in the female.

The *male genitalia* (Plate XVII, fig. 3) are rather of the *minutus* type. The distal segment of the *superior clasper* bears 4 spines, two apical and two almost at the junction of the terminal and middle third. These spines are stout and curved with spatulate ends, their lengths are slightly more than three-fourths that of the segment which carries them. The small deciduous spine is situated very distally on the segment, being distal to the points of origin of the large sub-apical spines. The intermediate appendages are of the usual type of the *minutus* group and are about three-fourths the lengths of the proximal segment of the superior clasper. The *intromittent organ* (Plate XVII, figs. 4 and 5) is well developed and terminates in a bluntly rounded end with a slit through which the genital filament was protruded in two specimens but not in the other. The shape of the organ resembles that figured by Adler and Theodor (1929) in the male of *P. minutus*. The *genital filament* has an obliquely truncated point, which is not swollen. The *pompetta* lies either in the 6th abdominal segment or is opposite the junction between the 6th and 7th segments. The *sub-genital lamellæ* (*cerci*) are about three-fourths the length of the inferior clasper.

TABLE I

Phlebotomus symesi n. sp. (♀)

	Structures	Lengths in mms. of specimens number —				Remarks, relative lengths, etc.‡
		1*	2†	3†	4†	
Body	Clypeus and head	0·400	0·400	0·385	0·400	
	Thorax	0·643	0·643	0·643	0·670	
	Abdomen proper	1·330	1·214	1·358	1·300	
	Superior clasper	0·170	0·143	0·143	0·143	
	Total length	2·54	2·40	2·53	2·50	= 1·41 to 1·53 × wing, 0·87 to 0·92 × leg
	Labium	0·250	0·235	0·250	0·250	$\frac{P}{L}$ = 3·0 to 3·08 $\frac{P}{E}$ = 3·4 to 3·6
	Epipharynx	0·222	0·204	0·222	0·216	
	Pharynx, length	0·190	0·180	0·180	0·180	= 2·4 to 2·57 × breadth
	Pharynx, breadth	0·075	0·072	0·075	0·075	

* Type female † Paratype females

‡ Compiled from the type and 7 paratype females

TABLE I—*contd*

	Structures	Lengths in mms of specimens number — 1*	2†	3†	4†	Remarks, relative lengths, etc‡
Antenna	Segment III	0·171	0·156	0·180	0·168	III > IV + V IV = V = VI
	Segment IV	0·087	0·087	0·090	0·090	IV + V + VI < XII to XVI
	Segment V	0·087	0·087	0·090	0·087	Antennal formula 2 / III to XV
	Segment VI	0·087	0·087	0·090	0·087	
	Segments XII to XVI	0·360	0·350	0·360	0·366	= 1·9 to 2·2 × IIIrd
	Total length	1·343	1·285	1·430	1·313	= 7·5 to 8·2 × IIIrd, 3·6 to 4·0 × XII to XVIth
Palp	Segment 1	0·045	0·039	0·045	0·039	Formula 1 2 (3, 4), 5
	Segment 2	0·105	0·102	0·105	0·105	1 + 2 = 3 = 4
	Segment 3	0·150	0·135	0·150	0·147	
	Segment 4	0·150	0·135	0·150	0·141	
	Segment 5	0·315	0·315	0·300	0·318	
	Total length	0·765	0·726	0·750	0·753	
Wing	Length	1·657	1·570	1·657	1·685	= 3·31 to 3·55 × breadth, = 0·57 to 0·62 × leg
	Breadth	0·500	0·457	0·500	0·500	$\frac{\alpha}{\beta}$ = 0·50 to 0·78 $\frac{\beta}{\gamma}$ = 1·15 to 1·58
	α	0·214	0·257	0·250	0·250	$\frac{\alpha}{\gamma}$ = 0·75 to 0·90 $\frac{\gamma}{\delta}$ = 0·26 to 0·53
	β	0·400	0·328	0·385	0·370	
	γ	0·285	0·285	0·285	0·285	$\frac{\alpha}{\delta}$ = 0·57 to 0·69 $\frac{\theta}{\varepsilon}$ = 2·10 to 2·46
	δ	0·071	0·128	0·100	0·114	
	ε	0·343	0·371	0·385	0·400	
	θ	0·830	0·786	0·828	0·483	$\frac{\alpha+\beta}{\theta}$ = 0·70 to 0·76 $\frac{\text{Wing}}{\theta}$ = 1·95 to 2·0
	π	0·114	0·085	0·071	0·143	
Hind leg	Femur	0·743	0·728		0·743	> ½ leg length, = ¾ tibia
	Tibia	1·000	0·971		1·000	> 2 × tarsus, segment 2
	Tarsus, segment 1	0·485	0·471		0·470	= 1/6th leg length
	Tarsus, segments 2 to 5	0·628	0·600		0·622	
	Total length	2·85	2·77		2·83	(Not including coxa and trochanter)
	Spermatheca, length	0·069	0·066	0·066	0·066	= 2 × breadth
	Spermatheca, breadth	0·034	0·039	0·042	0·034	

* Type female † Paratype females

‡ Compiled from the type and 7 paratype females

TABLE II

Phlebotomus symesi (♂).

	Structures	Lengths in mms of specimens number — 1 *	2 †	3 †	Remarks, relative lengths, etc
Body	Clypeus and head	0·370	0·328	0·343	
	Thorax	0·513	0·570	0·585	
	Abdomen proper	1·357	1·357	1·214	
	Superior clasper, segment 1	0·310	0·306	0·312	
	Total length	2·58	2·56	2·15	= 1·57 to 1·73 × wing length
	Labium	0·214	0·214	0·214	$\frac{P}{E}$ = 3·74 to 3·9 $\frac{P}{L}$ = 3·26 to 3·33
	Epipharynx	0·180	0·183	0·189	
	Pharynx, length	0·156	0·165	0·159	= 2·9 × breadth
	Pharynx, breadth	0·054	0·057	?	
Antenna	Segment III	0·183		0·186	III < IV + V
	Segment IV	0·102		0·102	IV = V = VI
	Segment V	0·102		0·105	
	Segment VI	0·100		0·105	Antennal formula $\frac{I}{III \text{ to } XV(?)}$
	Segments XII to XVI				
	Total length				
Palp	Segment 1	0·042	0·036	0·042	Formula 1, 2 (3, 4), 5
	Segment 2	0·096	0·096	0·100	1 + 2 = 3 = 4
	Segment 3	0·138	0·132	0·141	
	Segment 4	0·138	0·132	0·141	
	Segment 5	0·300	0·300	0·288	
	Total length	0·714	0·700	0·712	
Wing	Length	1·514	1·485	1·570	= 3·8 to 4·0 × breadth, = 0·57 × leg
	Breadth	0·400	0·370	0·400	
	α	0·214	0·200	0·185	$\frac{\alpha}{\beta}$ = 0·54 to 0·65 $\frac{\gamma}{\delta}$ = 1·15 to 1·20
	β	0·328	0·314	0·343	
	γ	0·285	0·270	0·285	$\frac{\alpha}{\gamma}$ = 0·65 to 0·75 $\frac{\alpha}{\delta}$ = 0·25 to 0·33
	δ	0·070	0·050	0·043	
	ε	0·357	0·343	0·320	$\frac{\alpha}{\varepsilon}$ = 0·58 to 0·60 $\frac{\alpha}{\theta}$ = 2·16 to 2·44
	θ	0·785	0·750	0·785	
	π	0·114	0·085	0·100	$\frac{\alpha+\beta}{\theta}$ = 0·67 to 0·70 $\frac{\text{Wing}}{\theta}$ = 1·96 to 2·00

* Type male † Paratype males

TABLE II—*contd*

	Structures	Lengths in mms of specimens number — 1*	2†	3†	Remarks, relative lengths, etc
Hind leg	Femur	0·685	0·670	0·685	= 1 leg length, = ⅔ tibia
	Tibia	0·930	0·900	0·957	= 2 × tarsus, segment 1
	Tarsus, segment 1		0·157		= 1/6th leg length
	Tarsus, segments 2 to 5		0·570		(Not including coxa and trochanter)
	Total length		2·60		
Genitalia	Superior clasper, segment 1	0·310	0·306	0·312	= 2·27 to 2·31 × seg 2, 1·12 to 1·14 × inf clasper
	Superior clasper, segment 2	0·135	0·135	0·135	
	Intermed append	0·240	0·243	0·246	= 0·87 to 0·90 × int clasp, 0·77 to 0·79 × sup clasp, seg 1
	Intromittent organ	0·183	0·192	0·192	= 0·76 to 0·79 × inter app
	Genital filament	0·021	0·000	0·030	(Length protruded)
	Inferior clasper	0·270	0·270	0·279	
	Sub-genital lamella	0·201	0·201	0·198	= 0·71 to 0·74 × inf clasper

* Type male

† Paratype males

Differential Diagnosis of *Phlebotomus symesi* sp n

The morphology of the buccal cavity and pharynx and the palpal formulæ are important diagnostic characters in both sexes In the male the situation of the spines on the distal segment of the superior clasper and the shape of the intromittent organ are also useful aids in identification

This species differs from the other recumbent-haired species recorded from Africa as follows —

(*a*) *P africanus* (Plate XX, figs 10—14) has a much more pointed and relatively narrower wing, its palpal formula is 1, 2, 3, 4, 5, the pigmented area and armatures of the buccal cavity and pharynx have a different morphology, the pharynx is more dilated posteriorly and the spermatheca runs into a narrow duct In the male also the intromittent organ is much narrower and ends in a sharper point (*cf* Adler and Theodor, 1929) and the non deciduous spine is much nearer the middle of the distal segment of the superior clasper

(*b*) *P minutes* and its variety *antennatus* have a palpal formula of 1, 2, 4, 3, 5, the pigmented area and the armatures of the buccal cavity and pharynx are very different, and the pharynx is almost flask-shaped In the male the spines on the distal segment of the superior clasper are apical

(*c*) *P similimus* has a palpal formula of 1, 2, 3, 4, 5, it has a much longer IIIrd antennal segment, in the wing α is greater than β, and its pharyngeal armature is much more distinctly toothed *

(*d*) *P bedfordi* has a palpal formula of 1, 2, 3, (4, 5), the pigmented area is more quadrilateral and the pharyngeal armature is markedly toothed *

(*e*) *P signatipennis* has narrow pointed wings, δ is negative, the antennal segments are relatively short, and its buccal and pharyngeal armatures more nearly resemble *P minutus* than *P symesi* *

(*f*) *P ingrami* has two of the four spines on the distal segment of the superior clasper situated about the middle of the segment

(*g*) *P fallax* has a very narrow, pointed wing and the spines on the distal segment of the superior clasper are all apical

(*h*) *P parroti* differs markedly in the morphology of the pigmented area and the buccal and pharyngeal armatures, the pharynx is much more dilated posteriorly

(*i*) *P squamipleuris* is distinguished by the scales on the pleuræ, the narrow pointed wing, the absence of geniculate spines on antennal segment IV, the presence of Newstead's spines on palpal segment 2, the different morphology of the buccal cavity and pharynx, and the spines on the distal segment of the superior clasper are all apical

The species appears to differ distinctly from any of the species of *Phlebotomus* from Africa yet described, and has been named *Phlebotomus symesi*

B Phlebotomus yusafi *sp n*

The descriptions given below are based on the study of two female and one male specimen collected by Dr C B Symes, at Mombasa, East Africa in November, 1929 The species is named *P yusafi* after my laboratory attendant, Mahomed Yusaf, who drew attention to its characteristic features while mounting the specimens

Phlebotomus yusafi (♀)

These specimens were mixed with those of *P symesi* and the only difference distinguishable in the dry state was the slightly smaller size and the fact that the integument of the sides of the thorax, instead of appearing yellowish-grey were rather of a light reddish-brown colour The dorsal abdominal hairs were recumbent

Appearances in Stained and Mounted Specimens

The measurements and ratios of the type and paratype females are given in Table III

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* The comparisons have been made with rough drawings made some years ago from specimens, chiefly types, which had not been specially mounted to show the buccal and pharyngeal armatures

The *total length* of the insect is 2 2—2 3 mms., being about equal to that of the hind leg The form of the cicatrices on the dorsum of the abdominal segments show that the species is a recumbent-haired one

The *buccal cavity* (Plate XVIII, fig 5) has a large dark pigmented area shaped rather like a peg-top with the point anteriorly The base of the area has a small projection backwards The teeth are well developed and number about 25 They are long and comparatively narrow, it is difficult to determine the character of their points against the dark pigmented area They are arranged in a curved line with the concavity posteriorly The *epipharynx* is shorter than in *P sumesi*, the ratio palp over epipharynx is 3 8

The *pharynx* (Plate XVIII, fig 6) is about 2 6 times as long as broad and its widest posterior portion is not quite twice as wide as the narrowest anterior part There is no marked posterior dilatation of this structure The pharyngeal armature is very well developed and consists of a series of long posteriorly directed spines The spines at the posterior part are slender and widely scattered, while the more anterior ones, arising from a dark-stained area, are much stouter and placed very close together These are very difficult to draw accurately, but are so closely placed together as to give the armature the appearance of longitudinal striations

The *antennæ* (Plate XVIII, figs 3 and 4) have paired geniculate spines on all segments from III to XV inclusive These spines are slender and of moderate length, those on the terminal segments overlapping the next more distal articulation Segment III is less than twice the length of segment IV, which is about one-fourth the combined lengths of segments XII to XVI The total length of the antenna is more than 9 times that of segment III and about 4 times that of segments XII to XVI The terminal segment is rather small and oval, and not so elongated as in *P sumesi*

The *palps* (Plate XVIII, fig 2) have a formula of 1, 2, 3, 4, 5 and the relative lengths of the segments is 2 2 5 5 7 9 10 19 6 The combined lengths of segments 1 and 2 equal that of segment 3, while the 5th is about twice the length of the 4th Newstead's spines are situated on the basal third of segment 3 and are about 25 in number

The *wing* (Plate XVIII, fig 1) is about 3 53—3 64 times as long as broad, is about two-thirds the length of β and the ratio δ over α is about 0 3—0 4 The proximal fork of the 2nd vein is very proximal to the fork of the 4th

The *spermatheca* (Plate XVIII, fig 9) is oval in shape and is about twice as long as broad, it opens into a wide duct The *post-genital plate* (Plate XVIII, fig 7) carries 3 or 4 spines The *furca* (Plate XVIII, fig 8) has ragged angles and the inner margins are inclined to curve ventrally, giving them a thickened appearance

Phlebotomus yusafi (♂)

The appearance in the dry state was very similar to that of the female

Appearance in Stained and Mounted Specimens

The ratios and measurements of the type specimen are given in Table IV

The *total length* of the insect is about 2 mms, which is approximately equal to that of the hind leg

The *buccal cavity* (Plate XIX, fig 5) shows a very small, irregularly rounded pigmented area and has about 14 small atrophied teeth The *pharynx* (Plate XIX, fig 5) shows little difference between the breadths of the anterior and posterior portions, the widest part being only about 1 2 times the narrowest Its length is about 3 times its breadth The armature is very poorly developed and consists of a few toothed ridges anteriorly and some lines of small teeth posteriorly The *epipharynx* is slender and the ratio palp over epipharynx about 4 28

The *antennæ* (Plate XIX, figs 3 and 4) bear single geniculate spines on all segments from III to XV inclusive These are comparatively small and slender Segment III is less than twice as long as segment IV The total length of the antenna is more than 9 times that of segment III and is about 4 times that of segments XII to XVI inclusive The distal end of segment III is about level with the distal end of the proboscis

The *palps* (Plate XIX, fig 2) have a formula of 1, 2, 3, 4, 5 and the relative lengths of the segments are 2 3 5 7 8 0 10 20 Segment 5 is twice the length of segment 4 Newstead's spines are on the proximal third of segment 3 and are about 5 in number

The *wing* (Plate XIX, fig 1) resembles that of the female The *hind leg* is about 1 6 times the length of the wing

The *male genitalia* (Plate XIX, fig 6) are of the *minutus* type The distal segment of the *superior claspei* which is about 3 times as long as broad, bears four curved spines all apically placed, some of these spines are longer than the segment which carried them The small non-deciduous spine is situated slightly distal to the middle of the segment (at about two-thirds) The *intermediate appendage* is of the usual *minutus* type but its end is smaller than that seen in *P symesi* The *intromittent organ* (Plate XIX, fig 7) is well developed with a broad, bluntly rounded end The *genital filaments* are slightly protruded The *pompetta* lies in the 7th abdominal segment The *sub-genital lamellæ* (*cerci*) are more than 4|5th the length of the inferior claspei

Differential Diagnosis of *Phlebotomus yusafi* sp n

The shape of the wing, the morphology of the buccal and pharyngeal armatures, the shape of the pharynx and spermatheca, the palpal formula and the position of the spines on the distal segment of the superior clasper, as well as the shape of the intromittent organ are useful diagnostic characters in the identification of this species

The species differs from the other recumbent-haired species recorded from Africa as follows —

(*a*) *P africanus* (Plate XX figs. 10—11) has a more pointed and relatively narrower wing, the pigmented area and the buccal and pharyngeal armatures are different, the pharynx is more dilated posteriorly, the spermatheca enters a narrow duct, and the intromittent organ is narrower and ends in a more acute point.

(*b*) *P minutus* and its variety *antennatus* have a palpal formula of 1, 2, 4, 3, 5, the pigmented area and the buccal and pharyngeal armatures are different, while the pharynx is widely dilated posteriorly.

(*c*) *P similimus* has a much longer IIIrd antennal segment, α is greater than β.

(*d*) *P bedfordi* has a palpal formula of 1, 2, 3, (4, 5) and a different buccal and pharyngeal armature.

TABLE III

Phlebotomus yusafi (♀)

	Structures	Lengths in mms. of specimens number —		Ratios, relative lengths, etc.
		1 *	2 †	
Body	Clypeus and head	0·357	0·357	
	Thorax	0·513	0·571	
	Abdomen proper	1·243	1·113	
	Superior claspers	0·113	0·130	
	Total length	2·30	2·20	= 1·46 to 1·56 × wing, = 0·93 to 1·02 × leg
	Labium	0·185	0·207	
	Epipharynx	0·171	0·165	$\frac{P}{L}$ = 3·03 to 3·37 $\frac{P}{E}$ = 3·8
	Pharynx, length	0·150	0·150	
	Pharynx, breadth	0·057	0·057	Length = 2·63 × breadth
Antenna	Segment III	0·105	0·130	III < IV + V
	Segment IV	0·063	0·081	IV + V + VI < XII to XVI
	Segment V	0·066	0·084	Antennal formula $\frac{2}{\text{III to XV}}$
	Segment VI	0·066	0·087	
	Segments XII to XVI	0·240	0·315	= 2·3 to 2·4 × IIIrd
	Total length	0·957	1·228	= 9·11 to 9·37 × IIIrd, = 4 × XII to XVIth
Palp	Segment 1	0·033	0·033	Palpal formula 1, 2, 3, 4, 5
	Segment 2	0·075	0·078	> ½ × 3rd
	Segment 3	0·111	0·117	1 + 2 ≤ 3
	Segment 4	0·144	0·147	
	Segment 5	0·288	0·282	= 2 × 4th
	Total length	0·651	0·627	

* Type female. † Paratype female.

TABLE III—*concld*

	Structures	Lengths in mms of specimens number — 1*	2†	Ratios, relative lengths, etc
Wing	Length	1·157	1·511	= 3·53 to 3·64 × breadth, = 0·63 to 0·65 × leg
	Breadth	0·100	0·128	
	α	0·207	0·220	$\frac{\alpha}{\beta}$ = 0·66
	β	0·314	0·285	$\frac{\beta}{\gamma}$ = 1·25 to 1·42
	γ	0·220	0·230	$\frac{\alpha}{\delta}$ = 0·93 to 0·96
	δ	0·064	0·085	$\frac{\delta}{\gamma}$ = 0·31 to 0·39
	ϵ	0·343	0·357	$\frac{\alpha}{\theta}$ = 0·60 to 0·62
	θ	0·743	0·743	$\frac{\epsilon}{\theta}$ = 2·08 to 2·16
	π	0·171	0·100	$\frac{\alpha+\beta}{\theta}$ = 0·70
				$\frac{\text{Wing}}{\theta}$ = 1·96 to 2·04
Hind leg	Femur	0·600	0·614	= ½ leg
	Tibia	0·770	0·843	= ½ leg
	Tarsus, segment 1	0·350	0·378	= 1/6th leg
	Tarsus segments 2 to 5	0·535	0·550	= ⅓ leg
	Total length	2·25	2·38	
	Spermatheca, length	0·042		= 2 × breadth
	Spermatheca, breadth	0·021		

* Type female † Paratype female

TABLE IV

Phlebotomus yusafi (♂)

	Structures	Lengths in mms of specimen	Ratios, relative lengths, etc
Body	Clypeus and head	0·300	
	Thorax	0·471	
	Abdomen proper	1·057	
	Superior clasper, segment 1	0·204	
	Total length	2·03	= 1·58 × wing, = 1·0 × hind leg
	Labium	0·170	
	Epipharynx	0·141	$\frac{P}{L}$ = 3·54 + $\frac{P}{E}$ = 4·28
	Pharynx, length	0·135	
	Pharynx, breadth	0·045	= 3 × breadth

TABLE IV—*contd.*

	Structures	Lengths in mms. of specimen	Ratios, relative lengths, etc
Antenna	Segment III	0·124	III < IV, V IV+V+VI < XII to XVI, IV + V + VI = 2 × III
	Segment IV	0·081	
	Segment V	0·082	
	Segment VI	0·084	
	Segments XII to XVI	0·285	= 2·31 × IIIrd
	Total length	1·157	= 9·1 × IIIrd, = 4 × XII to XVIth
Palp	Segment 1	0·030	Palpal formula 1, 2, 3, 4, 5
	Segment 2	0·072	> 4 × 3rd
	Segment 3	0·105	
	Segment 4	0·132	1 + 2 ≤ 3
	Segment 5	0·264	= 2 × 4th
	Total length	0·603	
Wing	Length	1·370	= 3·84 × breadth, = 0·64 × hind leg
	Breadth	0·357	
	α	0·143	$\frac{\alpha}{\beta} = 0·55$ $\frac{\beta}{\gamma} = 1·06$ $\frac{\alpha}{\gamma} = 0·59$
	β	0·257	
	γ	0·243	$\frac{\delta}{\alpha} = 0·20$ $\frac{\alpha}{\varepsilon} = 0·59$ $\frac{\theta}{\varepsilon} = 2·4$
	δ	0·028	
	ε	0·243	$\frac{\alpha + \beta}{\theta} = 0·65$ $\frac{\text{Wing}}{\theta} = 2·23$
	θ	0·614	
	π	0·086	
Hind leg	Femur	0·543	V, VI ¼ leg
	Tibia	0·730	V, VI ⅓ leg
	Tarsus, segment 1	0·357	
	Tarsus, segments 2 to 5	0·500	I–IV ¼ leg
	Total length	2·13	(Not including coxa and trochanter)
Genitalia	Superior claspers, segment 1	0·204	= 2·52 × seg. 2, = 1·19 × inf. clasper
	Superior clasper, segment 2	0·081	
	Intermed. append.	0·165	= 0·96 × inf. claspers, 0·80 × seg. 1
	Intromittent organ	0·123	= 0·74 × intermed. app.
	Genital filament	0·015	(Length protruded)
	Inferior clasper	0·171	
	Sub-genital lamellæ	0·147	= 0·86 × inf. clasper

(*e*) *P signatipennis* has narrow pointed wings, δ is negative, the antennal segments relatively short and its buccal and pharyngeal armatures different

(*f*) *P ingrami* has not got 4 apical spines on the distal segment of the superior clasper

(*g*) *P fallax* has a very narrow pointed wing, the palpal formula is 1, 2, (4, 3), 5, the distal segment of the superior clasper is long and narrow

(*h*) *P parroti* differs markedly in the morphology of its buccal and pharyngeal armatures, the pharynx is much more dilated posteriorly than in *P yusafi*

(*i*) *P squamipleuris* differs in the same points as given in the differential diagnosis of *P symesi*, except for the apical spines on the superior clasper in both species

(*j*) *P symesi* has a different palpal formula, the morphology of the pigmented area and the buccal and pharyngeal armatures is different, the spines on the superior clasper are not all apical

From the above diagnostic features it would appear that *P yusafi* is a new species which differs from those previously described from Africa

C Phlebotomus *sp*

The collection from Mombasa which contained *P symesi* and *P yusafi* also contained two male specimens which, while resembling the latter species, differed from it in several respects —the pharynx showed a marked posterior dilatation like that seen in *P africanus*, the posterior width being about twice that of the narrower anterior portion, the epipharynx was relatively shorter and stouter, the ratio palp over epipharynx being nearly 5, the distal segment of the superior clasper was about 4 times as long as broad and the small non-deciduous spine arose at about three-quarters the length of the segment

This species has not been named as it is hoped that later some females may be obtained

Phlebotomus in Sierra Leone

Although there are numerous records of *Phlebotomus* from the neighbouring colony of Gold Coast, there seem to be few from Sierra Leone Alcock (1912) reported *P minutus** from this area and Dalziel and Johnson (1915) records *P duboscqi* from the Sherbo district on the coast Except for these records we have been unable to discover any others

Colonel S R Christophers, CIE, FRS, IMS, has very kindly given me a specimen of *Phlebotomus* collected by him at Sierra Leone on 21st September,

* Alcock (1912) noted two varieties in the specimens received by him from Sierra Leone—dark specimens with broad wings and light specimens with narrow wings It seems very probable, in the light of more recent knowledge, that these were two different species, the former may have been *P freetownensis* and the latter *P africanus*

1928 This insect was captured in an empty grass shelter distant from other habitations, in heavy jungle near a stream on the outskirts of Freetown The specimen was at first considered to be a variety of *P africanus*, but so many points of difference have been found that it has been raised to specific rank as *P freetownensis*

Phlebotomus freetownensis sp n (♀)

The specimen was a large recumbent-haired species and as it has been preserved in spirit it was impossible to determine any colour peculiarities

Appearances in Stained and Mounted Specimen

The measurements, ratios, etc, of the type specimen are given in Table V, in which are also given similar details of a specimen of *P africanus* from Palestine for comparison

The *total length* of the insect is about 2 7 mms, which is also about the length of the hind leg

The *buccal cavity* (Plate XX, fig 7) has a triradiate pigmented area with thickened lateral arms, which show rounded ends, while the median or anterior one is pointed The armature consists of a line of narrow parallel teeth about 60 in number arranged in a curved line with the concavity posteriorly

The *pharynx* (Plate XX, fig 8) is flask-shaped, the widest posterior portion being about 2½ times as broad as the narrow anterior part, the length is nearly 3 times the greater breadth The armature consists of rows of very numerous fine teeth, the bases of which are distinctly separated from each other, at the widest part there are about 20 spines in a transverse row The *epipharynx* is short and stout, the ratio palp over epipharynx is nearly 5

The *antennæ* (Plate XX, figs 3 and 4) have paired geniculate spines on all segments from III to XV inclusive, these spines are short and stout Segment III is equal in length to that of IV and V together and much more than half that of segments XII to XVI inclusive, which are about equal to the combined lengths of segments IV, V and VI The terminal segment of the antenna is of medium length The total length is about 7 times that of segment III The end of segment III does not reach the end of the proboscis which is a little short of the end of segment IV

The *palp* (Plate XX, fig 2) has a formula of 1, 2, 3, 4, 5 and the relative lengths of the segments is 2 5 6 9 10 20 3 The combined lengths of segments 1 and 2 is much less than that of 3 Newstead's spines are situated on the basal third of segment 3 and are very numerous

The *wing* (Plate XX, fig 1) is broadly lanceolate and about 3 7 times as long as broad The ratio α over β is slightly greater than 1, while δ over α is 0 54

The *spermatheca* (Plate XX, fig. 9) is an elongated oval, the length being twice the breadth. It ends in a narrow duct (Type B of Adler and Theodor, 1927). The inner margins of the *furca* (Plate XX, fig. 6) are thickened.

TABLE V

Phlebotomus freetownensis (♀)

	Structures	Lengths in mms. of specimens number —		Ratios, relative lengths, etc ‡
		1 *	2 †	
Body	Clypeus and head	0·371	0·313	
	Thorax	0·657	0·570	
	Abdomen proper	1·530	0·885	
	Superior clasper, segment 1	0·113	0·100	
	Total length	2·7	1·9	= 1·3 (1·17) × wing length, 1·02 (0·81) × leg
	Labium	0·214	0·170	$\frac{P}{L}$ = 3·9 (3·15) $\frac{P}{E}$ = 5·04 (4·36)
	Epipharynx	0·165	0·123	
	Pharynx, length	0·162	0·126	
	Pharynx, breadth	0·057	0·045	= 2·84 (2·8) × breadth
Antenna	Segment III	0·192	0·135	III = IV + V (III < IV + V)
	Segment IV	0·093	0·072	IV + V + VI ≦ XII to XVI
	Segment V	0·096	0·075	
	Segment VI	0·096	0·075	Antennal formula $\frac{2}{\text{III to XV}}$
	Segments XII to XVI	0·297	0·240	
	Total length	1·357	1·050	= 7·06 (7·8) × IIIrd, = 4·55 (4·40) × XII to XVIth
Palp	Segment 1	0·036	0·030	Formula 1, 2, 3, 4, 5
	Segment 2	0·100	0·066	1 + 2 << 3 (1 + 2 < 3)
	Segment 3	0·160	0·105	
	Segment 4	0·177	0·111	
	Segment 5	0·360	0·225	= 2 × 4th
	Total length	0·838	0·537	

* Type of *P. freetownensis* (♀)

† *P. africanus* from Palestine for comparison

‡ The ratios of *P. africanus* are given in brackets

TABLE V—*concld*

	Structures	Lengths in mms of specimens number — 1*	2†	Ratios relative lengths, etc‡
Wing	Length	1·857	1·611	3·70 (4·25) × breadth, = 0·7 × leg
	Breadth	0·500	0·480	
	α	0·357	0·170	$\frac{\alpha}{\beta}$ = 1·06 (0·46) $\quad \frac{\beta}{\gamma}$ = 1·17 (1·37)
	β	0·331	0·370	
	γ	0·285	0·270	$\frac{\alpha}{\gamma}$ = 1·25 (0·63) $\quad \frac{\delta}{\alpha}$ = 0·54 (0·65)
	δ	0·193	0·011	$\frac{\alpha}{\varepsilon}$ = 0·72 (0·55) $\quad \frac{\theta}{\varepsilon}$ = 1·95 (2·71)
	ε	0·193	0·300	
	θ	0·961	0·811	$\frac{\alpha+\beta}{\theta}$ = 0·72 (0·66) $\quad \frac{\text{Wing}}{\theta}$ = 1·92 (2·00)
	—	0·157	0·111	
Hind leg	Femur	0·771	0·643	> 1 leg
	Tibia	0·813	0·785	= 1 leg, = (>) 2 × seg 1 tarsus
	Tarsus, segment 1	0·128	0·357	
	Tarsus, segments 2 to 5	0·611	0·513	
	Total length	2·65	2·32	(Not including coxa and trochanter)
	Spermatheca, length	0·075	0·015	= 2 × breadth
	Spermatheca breadth	0·037	0·021	

* Type of *P freetownensis* (♀)

† *P africanus* from Palestine for comparison

‡ The ratios of *P africanus* are given in brackets

Differential Diagnosis of *P freetownensis* sp n (♀).

The morphology of the buccal cavity and pharynx differ markedly from those described in any other African species except *P africanus*, from which it differs in the following features —

P africanus (Plate XX, figs 10—14) is a much smaller insect in every respect, the pigmented area has not such a marked concavity posteriorly, the buccal teeth are only about 40 in number, the spines on the pharyngeal armature are larger and fewer and the transverse rows only carry about 10 teeth, the ratio palp over epipharynx is smaller, the total length of the antenna is nearly 8 times segment III and the latter segment is shorter than the combined lengths of IV and V, the relative lengths of the palpal segments are 2 6 5 9 9 1 10 19, segments 1 and 2 of the palp are relatively longer, the

wing is narrow and pointed, its length being more than 4 times its breadth, — α is only about half the length of β and δ is very small

The only other species likely to be confused with this is *P similimus*, but the latter has a much longer IIIrd antennal segment, which forms about one-sixth of the total length of the antenna, and the proximal fork of the 2nd wing vein is about level with the fork of the 4th

The insect would therefore appear to belong to a new species which has been named *Phlebotomus freetownensis*

Summary

(1) The chief records of the distribution of *Phlebotomus* in Africa have been summarized

(2) Two new species, *P symesi* and *P yusafi*, have been described from East Africa, and one new species, *P freetownensis*, from Sierra Leone *

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* When this paper was almost ready for the press there appeared in the January number of the *Rev Applied Entomology* (XVIII, B, 1, pp 4-5) notice of a paper by Adler, Theodor and Parrot (1929) (*Rev Zool Bot Afr*, XVIII, 1, pp 72—89) giving details of *P africanus*, *P similimus* and *P ingrami* from the Belgian Congo as well as of three new species, *P schwetzi*, *P schoutedeni* and *P collarti* from that area In the same journal (pp 90-91) Parrot (1929) also records a new species, *P ghesquierei*, from that locality Unfortunately the originals of these papers are not available, so I am unable to determine the relation of these species to those described above

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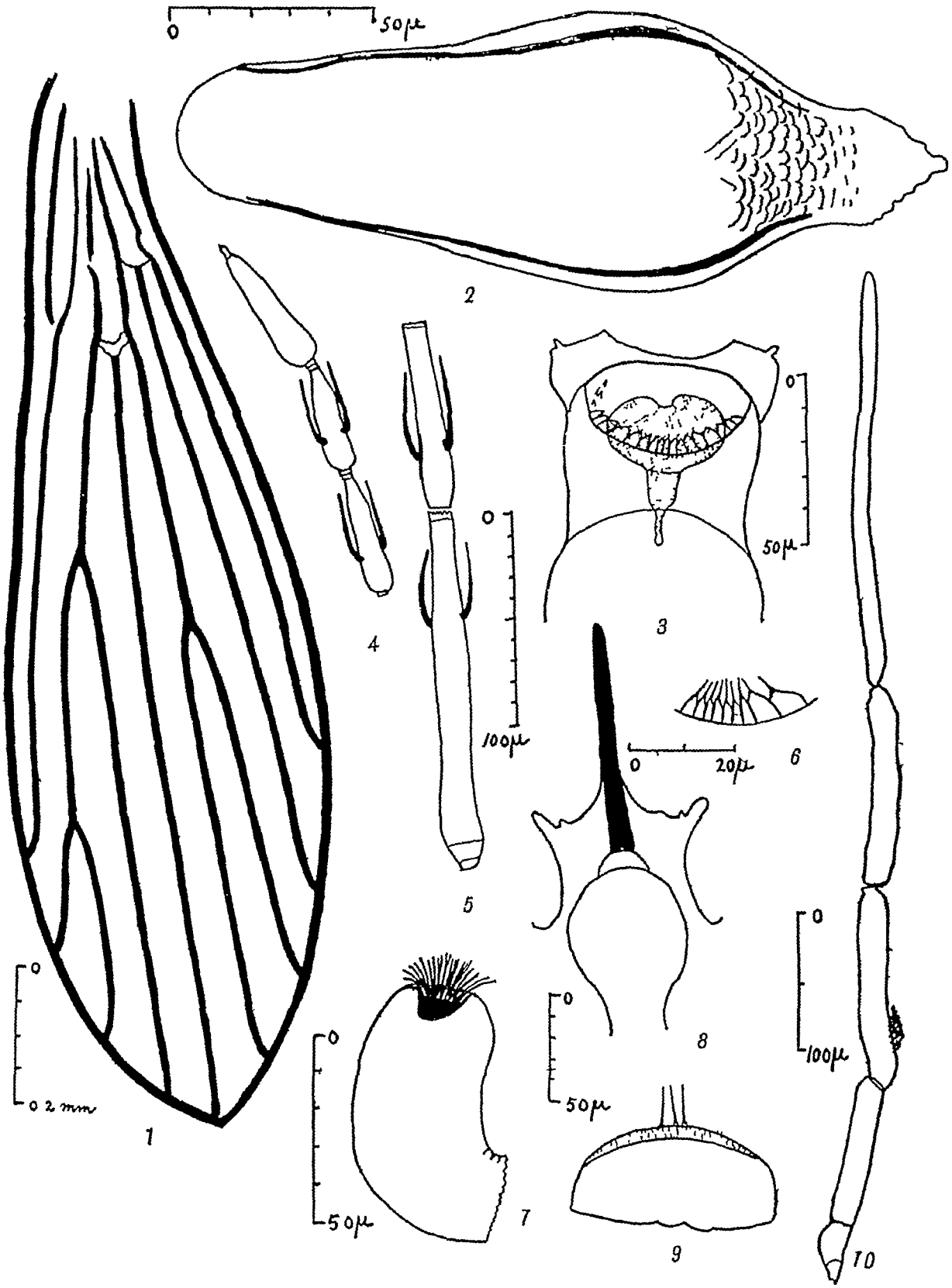
Explanation of Plate XVI

Phlebotomus symesi (♀)

Fig 1 Wing
„ 2 Pharynx
„ 3 Buccal cavity
„ 4 Antennal segments XIV, XV and XVI
„ 5 Antennal segments III and IV
„ 6 Some teeth of buccal armature showing their points
„ 7 Spermatheca
„ 8 Furca
„ 9 Post-genital plate
„ 10 Palp showing position of Newstead's spines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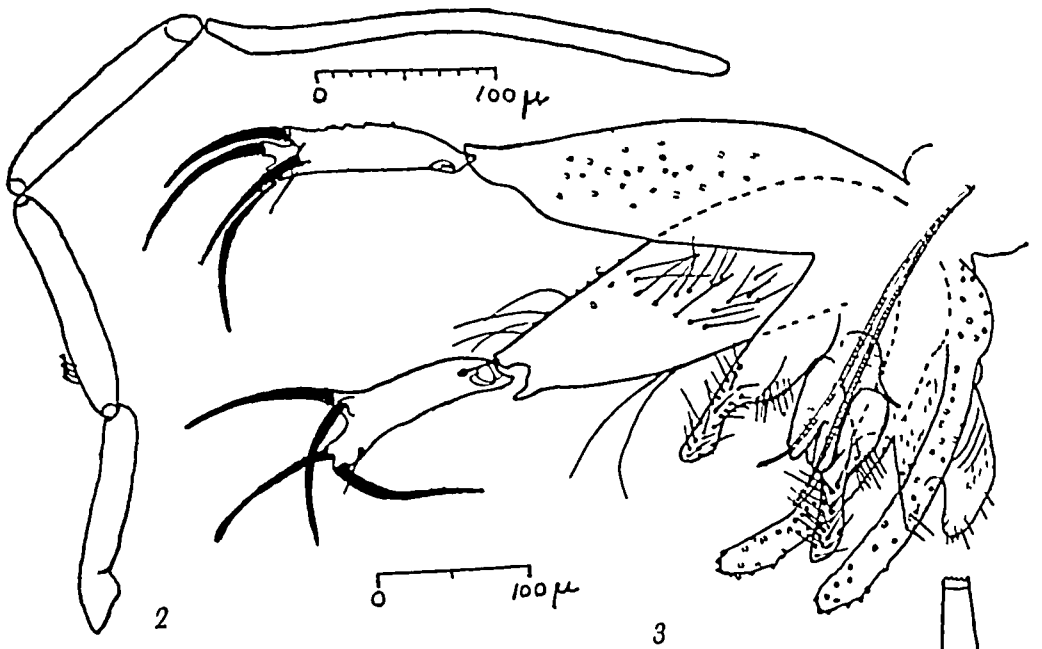

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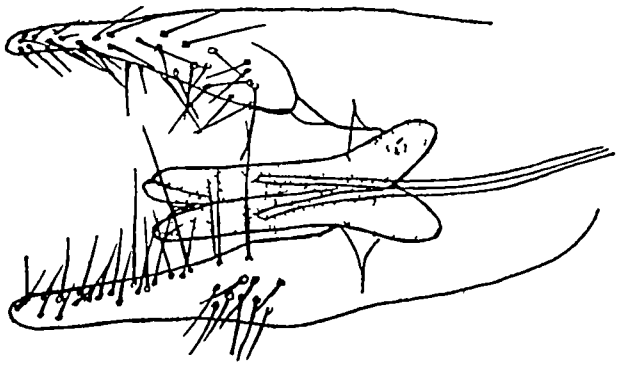

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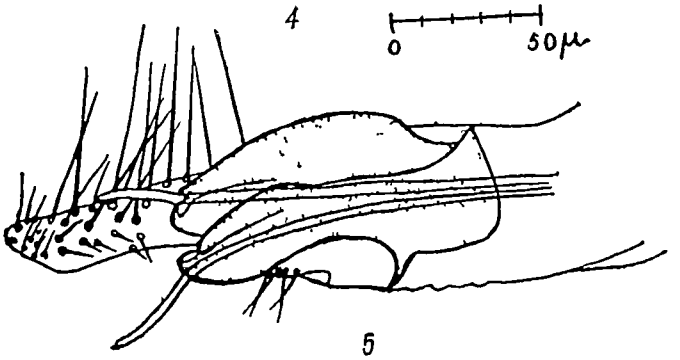

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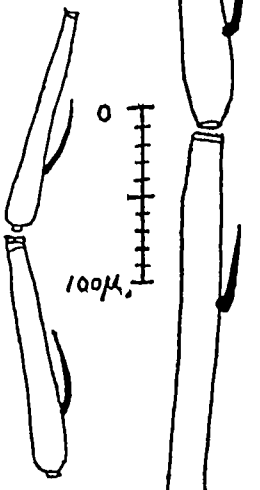

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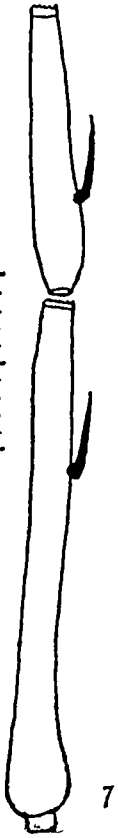

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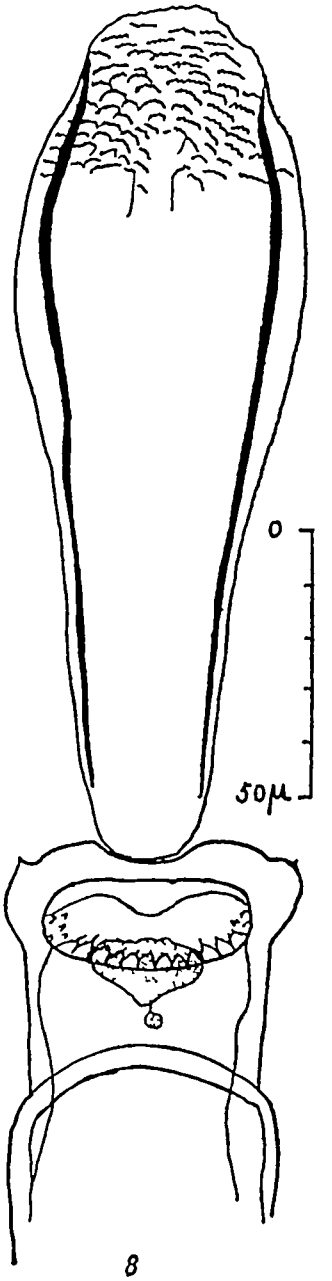

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Explanation of Plate XVII

Phlebotomus symesi (♂)

Fig 1 Wing
„ 2 Palp showing position of Newstead's spines
„ 3 Male genitalia
„ 4 Intermediate appendage and intromittent organ viewed from above
„ 5 Intermediate appendage and intromittent organ seen from side
, 6 Antennal segments XI and XII
„ 7 Antennal segments III and IV
8 Pharynx and buccal cavity

Explanation of Plate XVIII

Phlebotomus yusafi (♀)

Fig 1 Wing
„ 2 Palp showing position of Newstead's spines
„ 3 Antennal segments XII—XVI
„ 4 Antennal segments III and IV
„ 5 Buccal cavity
„ 6 Pharynx
„ 7 Post-genital plate
„ 8 Furca
„ 9 Spermatheca

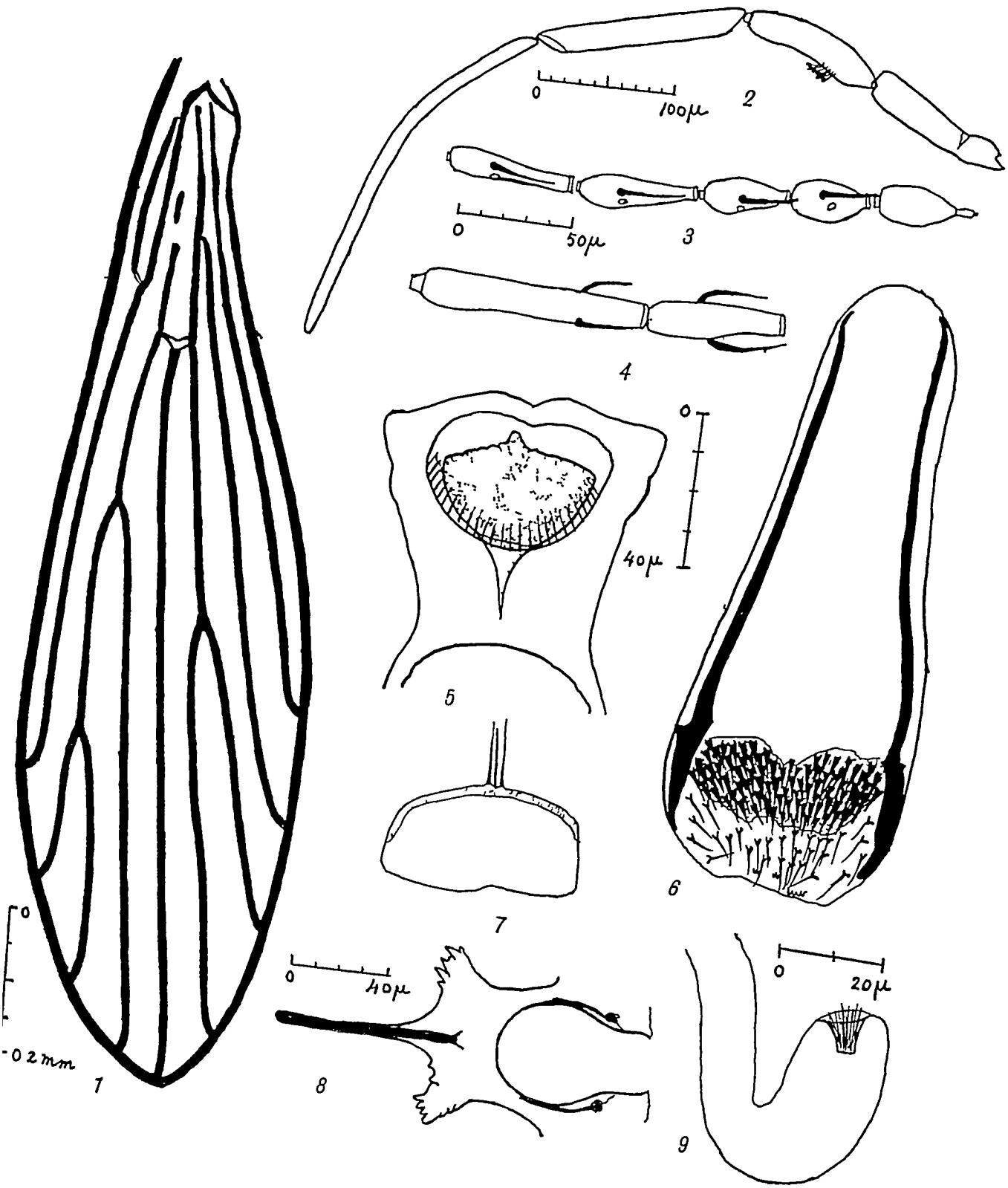
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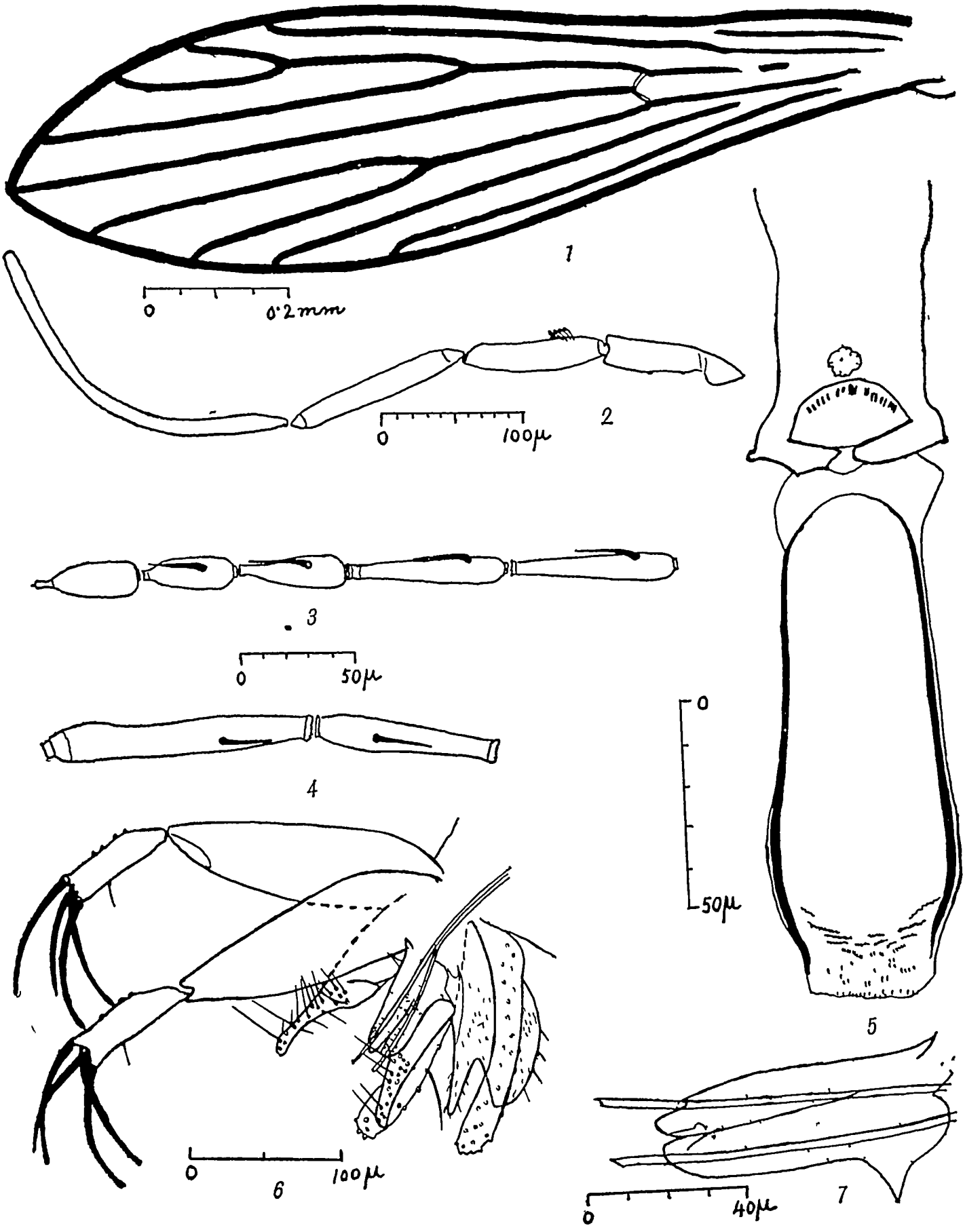
PLATE XIX



Explanation of Plate XIX

Phlebotomus yusafi (♂)

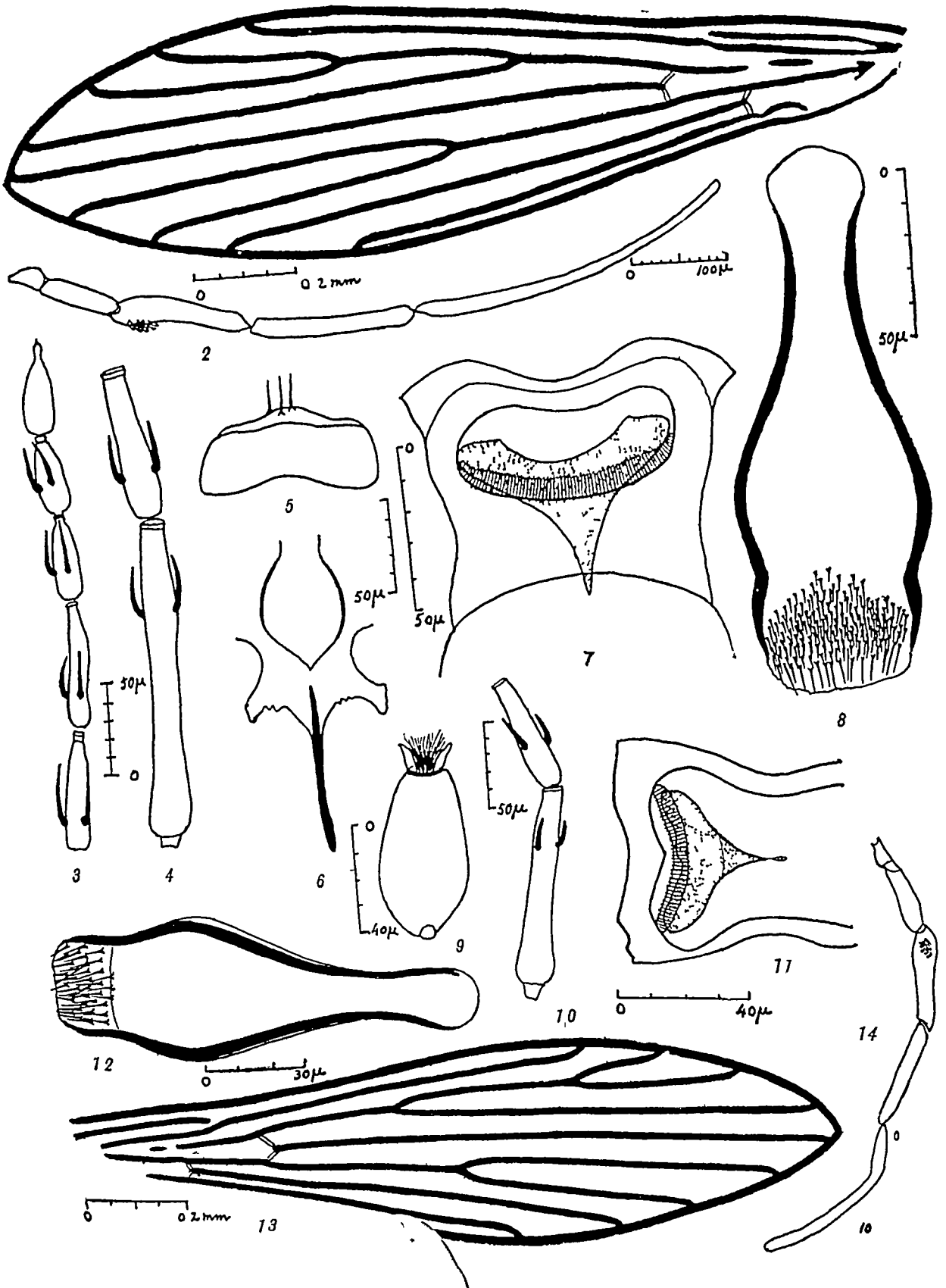
Fig 1 Wing
„ 2 Palp showing position of Newstead's spines
„ 3 Antennal segments XII—XVI
„ 4 Antennal segments III and IV
„ 5 Buccal cavity and pharynx
„ 6 Male genitalia
„ 7 Lateral view of intromittent organ showing protruded genital filaments

Explanation of Plate XX

Phlebotomus freetownensis (♀) and *Phlebotomus africanus* (♀)

Fig 1 Wing of *P freetownensis*
„ 2 Palp of *P freetownensis*
„ 3 Antennal segments XII—XVI of *P freetownensis*
„ 4 Antennal segments III and IV of *P freetownensis*
„ 5 Post-genital plate of *P freetownensis*
„ 6 Furca of *P freetownensis*
„ 7 Buccal cavity of *P freetownensis*
„ 8 Pharynx of *P freetownensis*
„ 9 Spermatheca of *P freetownensis*
„ 10 Antennal segments III and IV of *P africanus* from Palestine
„ 11 Buccal cavity of *P africanus*
„ 12 Pharynx of *P africanus*
„ 13 Wing of *P africanus*
„ 14 Palp of *P africanus*

PLATE XX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NOTES ON SOME INDIAN SPECIES OF THE GENUS *PHLEBOTOMUS*

Part XXV

PHLEBOTOMUS MAYNEI N SP

BY

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[Received for publication, February 10, 1930]

A NUMBER of specimens of *P squamipleuris* Newstead, which had been attracted by light, were captured on 28th July, 1927, in a room at Saharanpore in the United Provinces When these had been mounted, one male of a hitherto undescribed species of sandfly was discovered among them As the species was only recognized after staining and mounting in balsam, no description of the insect in the fresh state can be given The numerous cicatrices on the dorsum of the abdominal segments, however, show that it belongs to the 'erect-haired' group of the genus *Phlebotomus*

Phlebotomus maynei n sp (♂)

The attached Table gives the measurements and relative lengths of the different parts of the specimen *

The insect is of medium size, being about 2 33 mm in length The abdomen proper forms about half the total length

The *Pharynx* (Plate XXI, fig 3) is three times as long as broad The armature is composed of a series of oblique ridges radiating from the middle

* Although the elaborate tables of measurements originally proposed by França and Parrot (1920) have been replaced to a great extent by simpler and more accurate means for the diagnosis of the species of this genus, yet it seems to me that, in the case of descriptions of new species at least, such measurements should always form part of the permanent record of the species, to eliminate as far as possible any doubt as to the insect described, even if the type specimen is lost in the future (Sinton, 1929)

line and ending in loops laterally No pigmented area is present and the buccal cavity is unarmed, except for the few small scattered teeth usually seen in members of this group of *Phlebotomus*

The *Antennæ* (Plate XXI, figs 4, 5, 6) have very long paired geniculate spines on all segments from the IIIrd to XVth inclusive These spines on the more proximal segments tend to be asymmetrical and their ends surpass the next inter-segmental junction The IIIrd segment is longer than the combined lengths of segments IV and V but is shorter than that of segments XII to XVI

The *Palps* (Plate XXI, fig 2) are characterized by a very short 4th segment, which is only half as long as the combined lengths of segments 1 and 2 Newstead's spines are about 12-13 in number and scattered along the middle third of segment 3

The *Wings* (Plate XXI, fig 1) resemble those of *P argentipes,* but the origin of the 3rd vein is much closer to the termination of the subcostal vein than in that species (*cf* Sinton, 1923, Sinton, 1925)

The *Hind Leg* has a femur equal in length to that of tarsal segments 2 to 5, forming about 1/5th the total length of the leg

The *Male Genitalia* (Plate XXI, fig 7) are very characteristic The distal segment of the superior clasper (Plate XXI, figs 8, 9) is rather pyriform in shape and resembles that of *P sergenti* var *alexandri* (*P sergenti* var Newstead, 1921) It carries 4 spines—one terminal arising from a longish process and one subterminal arising from a shorter tubercle These spines are both very stout with spatulate ends and measure about 84μ in length The other two spines take origin from two low tubercles situated on the inner side of the segment near the junction of its middle and proximal thirds These spines are much more slender than the distal ones and measure about 90μ in length The proximal segment of this clasper is long and slender and carries an oval collection of long hairs on the inner side at the junction of the proximal and middle thirds, resembling those seen in *P major* and *P chinensis* There is no tuft of hairs arising from a tubercle at the base of the segment, as in *P sergenti* and its varieties

The intermediate appendage (Plate XXI, fig 10) is trilobed and closely resembles that of *P argentipes,* except that the small ventral lobe carried two long curved spines, not four short ones (*cf* Sinton, 1923, Sinton, 1925) As in *P argentipes* and *P newsteadi,* two long stout spines arise at the junction of this appendage with the intromittent organ and run backward on either side of the latter structure

The intromittent organ is well developed with an oval bifid end The genital filaments are not protruded No specially characteristic features were noted in the morphology of the inferior claspers and subgenital lamellæ The pompetta lies in the abdomen opposite the junction of the 4th and 5th segments

TABLE

Phlebotomus mayneri n. sp. (♂)

	Structure	Lengths in mms.	Relative lengths, formulæ, etc.
Body	Clypeus and head	0·330	
	Thorax	0·513	
	Abdomen proper	1·200	= ½ body length
	Superior clasper, segment 1	0·255	
	Total length	2·33	= 1·35 × wing length, = 0·68 × hind leg
	Labium	0·185	
	Epipharynx	0·170	
	Pharynx, length	0·117	= 3 × breadth
	Pharynx, breadth	0·039	
Antenna	Segment III	0·285	III > IV + V IV = V = VI
	Segment IV	0·120	Antennal formulæ $\frac{2}{\text{III to XV}}$
	Segment V	0·120	
	Segment VI	0·120	IV + V + VI < XII to XVI
	Segments XII to XVI	0·161	= 1·62 × IIIrd
	Total length	1·930	= 6·75 × IIIrd, = 1·15 × XII to XVIth
Palp	Segment 1	0·036	Palpal formulæ—1, 4 2 3, 5
	Segment 2	0·072	Relative lengths—6·6, 13·3, 22·2, 10, 29
	Segment 3	0·120	
	Segment 4	0·051	= ½ × 1 + 2 $\frac{P}{L}$ = 2·37 $\frac{P}{E}$ = 2·58
	Segment 5	0·156	
	Total length	0·438	
Wing	Length	1·730	= 3·56 × breadth, = 0·50 × hind leg
	Breadth	0·185	
	α	0·400	$\frac{\alpha}{\beta}$ = 1·64 $\frac{\beta}{\gamma}$ = 1·13 $\frac{\alpha}{\gamma}$ = 1·87 $\frac{\delta}{\alpha}$ = 0·14
	β	0·243	
	γ	0·214	
	δ	0·057	$\frac{\alpha}{\epsilon}$ = 0·75 $\frac{\beta}{\epsilon}$ = 0·46 $\frac{\theta}{\epsilon}$ = 1·57 $\frac{\alpha+\beta}{\theta}$ = 0·77
	ϵ	0·530	
	θ	0·828	$\frac{\text{Wing}}{\theta}$ = 2·08
	τ	0·085	
Hind leg	Femur	0·685	= 1/5th leg length, = tarsus, segments 2 to 5
	Tibia	1·285	
	Tarsus, segment 1	0·770	
	Tarsus, segments 2 to 5	0·685	
	Total length	3·43	(Not including coxa and trochanter)
Genitalia	Superior clasper, segment 1	0·255	= 2·75 × segment 2, = 1·46 × intermediate appendage, = 1·09 × inferior clasper
	Superior clasper, segment 2	0·093	
		0·174	= subgenital lamellæ
	Intermittent organ	0·135	
	Inferior clasper	0·234	= 1·35 × intermediate appendage
	Subgenital lamellæ	0·180	
	Pompetta, length	0·150	

Identity of the Specimen

The male genitalia of this species is absolutely distinct from that of any of the other species of *Phlebotomus* described up to the present It is evidently a new species which I have great pleasure in naming *Phlebotomus mayneı* after my colleague, Dr Bruce Mayne, in whose rooms it was captured

Diagnostic Characters

In fresh specimens the very short 4th palpal segment differentiates this species from the other large members of the 'erect-haired' group, except *P argentipes,* from which it can be distinguished by the fact that the termination of the subcostal vein of the wing approximates the origin of the 3rd vein and is not widely separated from it as in *P argentipes*

In mounted specimens, in addition to the above features, the species is easily differentiated by its characteristic male genitalia, which are best described as resembling those of *P argentipes,* except that the distal segment of the superior claspei is like that of *P sergenti* var *alexandri* The pharyngeal armature also distinguishes it from the other members of the 'erect-haired' group (*cf* Sinton and Barraud, 1928)

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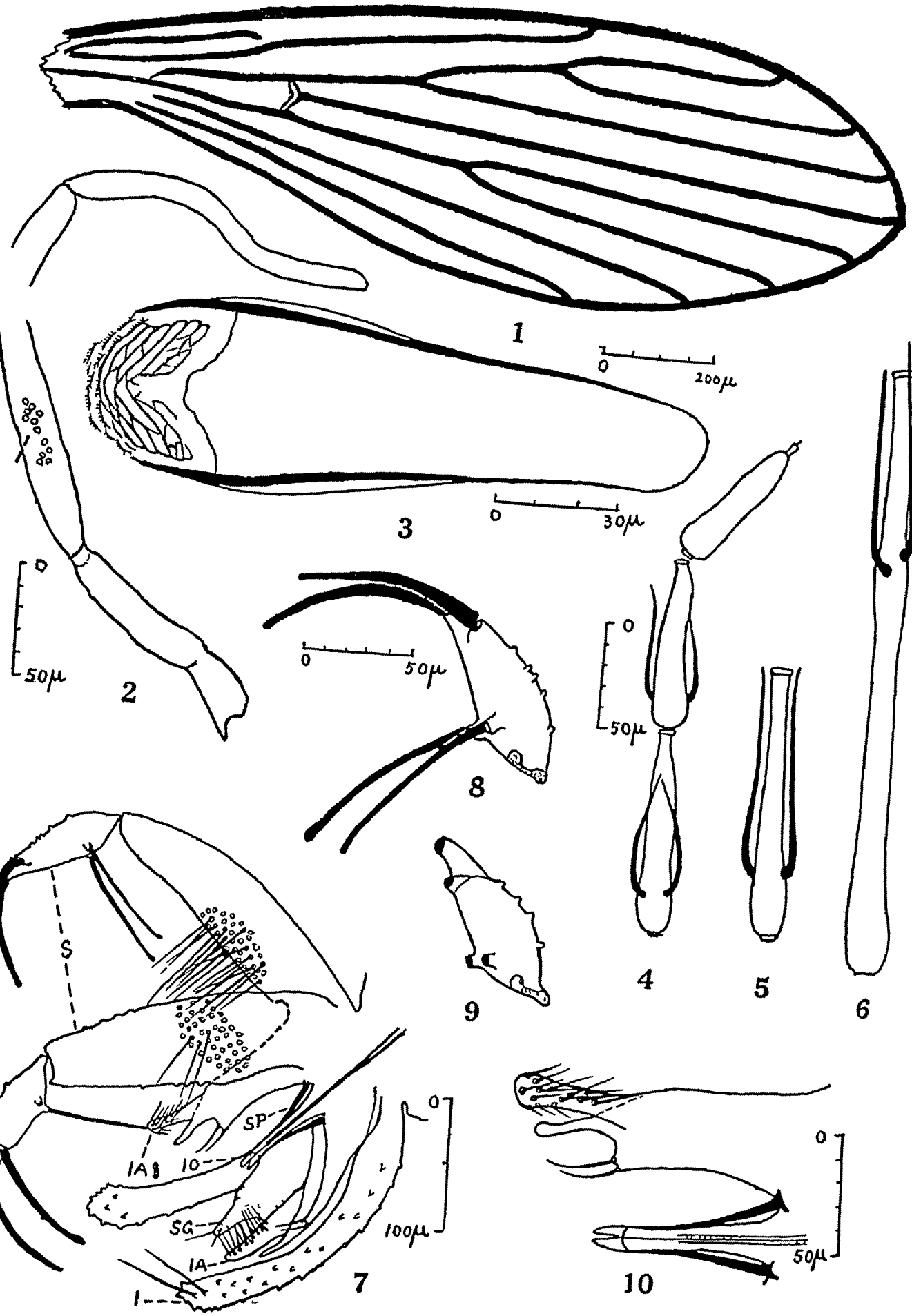
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Explanation of Plate XXI

Phlebotomus maynei (♂)

Fig 1 Wing
„ 2 Palp 'N'—Newstead's spines
„ 3 Pharynx showing armature
„ 4 Segments XIV to XVI of antenna
„ 5 Segment VII of antenna
„ 6 Segment III of antenna
„ 7 Male genitalia 'S,' superior clasper, 'I,' inferior clasper, 'SG,' subgenital lamellæ, 'IA,' intermediate appendage, 'IO,' intromittent organ, 'SP,' spines
„ 8 Distal segment of superior clasper
„ 9 Distal segment of superior clasper, after removal of spines
„ 10 Intermediate appendage and intromittent organ

A FIELD METHOD FOR THE ESTIMATION OF THE SALINITY OF THE WATER IN MOSQUITO-BREEDING PLACES

BY

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AND

N D KEHAR, M SC

(Malaria Survey of India, Kasauli)

[Received for publication, February 18, 1930]

THE bionomics of mosquitoes form such an important factor in anti-malarial work that it is essential that all information on the subject should be as accurate as possible consistent with practicability In reports on the presence or absence of mosquito larvæ in certain collections of water one often comes across such vague descriptive terms as 'brackish,' 'salty,' 'very saline,' etc These terms are really matters of personal opinion and give no accurate information for comparative purposes The absence of more precise information seems mainly due to the difficulties involved in carrying back large numbers of water samples to the laboratory for analysis If the estimations of salinity could be made on the spot by some simple and compact apparatus, such objections would be eliminated and valuable information would be obtained, which would be comparable in all instances

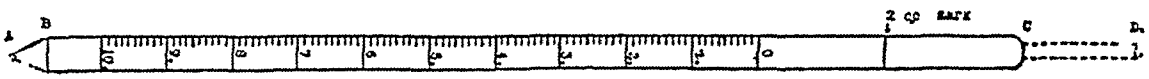
The method described below was devised to overcome this difficulty The apparatus needed can easily be prepared in most laboratories and occupies very little space in the field equipment

Apparatus and Materials

The following apparatus is needed —(*a*) A special tube made from a 10 c c graduated pipette, (*b*) two standard solutions of silver nitrate and (*c*) a solution of potassium chromate

(*a*) The *special tube* (*see* Text-figure) is prepared from a 10 c c delivery pipette graduated in 1/10th c cs The method of preparation is as follows —

The delivery point A of the pipette is cut off at B with a file or glass-knife The other end of the pipette is then closed at C by cutting off the mouth-piece D with the flame of a blow-pipe and sealing it at the same time When the tube has cooled, alcohol is poured into it up to the zero graduation and the amount taken is measured If this is not an exact number of cubic centimetres, the alcohol is poured off and, after thoroughly drying the tube, the end C is again heated in the flame to decrease its size until the ungraduated



Text-figure

portion of the tube holds an exact number of cubic centimetres of fluid In the pipettes used by us the amount is 4 c c, as this is almost exactly the quantity contained after the mouth-piece is sealed off A permanent mark is also made on the ungraduated portion at the 2 c c point

(*b*) *Standard solutions of silver nitrate* are prepared of such a strength that in the weaker solution 1 c c is capable of precipitating exactly 2 mg of chlorine, while in the stronger solution 1 c c will precipitate 10 mg of chlorine The weaker solution is made by dissolving 9 58 grms of pure recrystallized silver nitrate in distilled water and making the solution up to a litre, the stronger solution contains 47 9 grms to the litre These solutions are kept in brown coloured bottles

(*c*) *A saturated solution of potassium chromate* is prepared in distilled water

Technique

This is the ordinary water-analysis technique for the estimation of chlorides in water, in which potassium chromate is placed in the water sample as an indicator, and when silver nitrate is added no permanent change of the yellow colour takes place until all the chlorine present has been changed to silver chloride, after which the uncombined silver unites with the chromate to form its silver salt which gives a permanent reddish coloration The method here described can be used either in the field or the laboratory and only requires a few minutes to carry out

In the field one carries the special tube, two brown drop-bottles holding 100 c c or more of each of the silver solutions and a small drop-bottle of the chromate solution When a sample of water is to be tested, the tube is washed out with the water and then filled exactly to the zero mark Two drops or so of the chromate solution are mixed with the water and the silver nitrate solution carefully added a few drops at a time, the tube being well shaken after each addition This procedure is continued until the yellow colour just disappears and a permanent faint red coloration remains The

amount of silver solution used is then read off the graduations of the tube to the nearest 1/10th c.c.

If the weak silver solution is used the parts of chlorine per 100,000 are calculated as follows —

$\frac{100}{a} \times \frac{2b}{1}$ = parts of chlorine per 100,000, while if the stronger solution is used the formula will be —

$\frac{100}{a} \times \frac{10b}{1}$ = parts per 100,000, when 'a' is the number of c.cs of water tested and 'b' is the number of c.cs silver solution used.

If it is desired to give the result as potential sodium chloride the parts of chlorine are multiplied by 1·65.

The proportion of chlorine in different waters may vary from almost none in rain or peaty waters up to 2,000 parts per 100,000 in sea-water, or even more in salt-water pools which have been concentrated by the sun.

If the ungraduated portion of the tube contains 4 c.c. water, each graduation on the tube equals 5 parts of chlorine per 100,000, if the weak silver solution is used. There is thus a range from 5 to 500 parts per 100,000, and if a lower range is required 8 or even 12 c.c. of water may be placed in the tube. This range should meet most requirements in inland waters but if one is working near the seashore the stronger silver solution may be required. This solution has a range from 25 to 2,500 parts per 100,000 in steps of 25 parts, if 4 c.c. of water is used, while if only 2 c.c. are taken the range is from 500 to 5,000 in steps of 50 parts.

SOME ASPECTS OF THE BEHAVIOUR OF CINCHONA ALKALOIDS TOWARDS LIVING CELLS *

BY

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[Received for publication, March 20, 1930]

SINCE the time of Graham, in the middle of the last century, the penetration of electrolytes into gels has been studied by many workers, while in more recent years Von Furth and Bubanovic (1918) and Stiles (1919) have specially investigated this subject The technique used by the latter workers was similar to that employed by Lodge (1886) for comparing the mobilities of ions The chief feature in this method is that the diffusion of the electrolyte into the gel is estimated by means of an indicator mixed with the gel substance

Although the penetration of many substances has been studied by different workers, yet no research seems to have been recorded on the penetration and behaviour of any of the cinchona alkaloids in colloidal systems and living cells in the presence of different electrolytes Such an investigation might shed light on the mode of action of these drugs which play a very important part in the treatment of malaria

The present research was undertaken to investigate some of the physico-chemical factors influencing the diffusion and permeability in colloidal systems and in certain plant cells, when one of the quinine salts is brought in contact with them And also to find out whether the presence of some of those electrolytes, which constitute an important part of the inorganic salts of the body fluids and blood plasma, have any marked effect on the above phenomena

The cell contents and other body fluids have all been proved to be colloidal in nature by Czapek (1911) and Stiles (1920), and as gelatin and agar-agar are typical colloids (Svedberg, 1924), these gels have been selected for the present investigation on the supposition that the phenomena occurring in them will be common to all colloidal systems These phenomena might reasonably be expected to apply to body cells and tissues also, and thus help in elucidating the mechanism of diffusion and permeability of certain substances in the living cells

* A paper read before the Medical Section, Indian Science Congress, 1930

From the point of view of Physical Chemistry, the cell contents consist of a collection of colloids, crystalloids, electrolytes and non-electrolytes dissolved or suspended in water, in lipoids or in each other The outer surface resembles a thin semi-permeable film, the plasma membrane, which functions as a protective and a selective layer, and which according to Chambers (1915) is responsible for the semi-permeable properties of the protoplasm We may regard it as a delicate diffusion membrane, situated at the surface of the cell, allowing a ready passage of water, but offering a varying amount of resistance to the passage of various crystalloids and colloids, the degree of impermeability being dependent on a number of physico-chemical factors The inner portions of the cell would appear to consist of a mass of well-developed heterogenous colloidal emulsions

Experiments on dead cells cannot be of much help, because when a cell dies, it passes from a state of emulsoid sol into one of emulsoid gel, thereby changing in its character and reactions Besides this, other profound structural changes occur in the protoplasm, with respect to coagulation, physical consistency, and tensile strength One of the most remarkable differences between a dead and a living cell is that the liquid of the former mixes freely with the surrounding watery solutions, while the contents of the latter do not behave in this manner It may be, that the compounds which during life are kept apart by barriers of some kind become free to interact when the protoplasmic structure alters at death The multinucleated living cells of *Chara* (Characæ), a water alga, were selected for these experiments, because these cells form a very suitable object for the study of protoplasmic changes in life and death

I Penetration

1 *Technique*

(*a*) Preparation of gels —The colloidal systems selected for studying the penetration of quinine salts were gelatin and agar gels The gelatin gels were prepared by allowing a 10 per cent emulsion of Merck's pure brand of gelatine to cool and set, and agar gels by dissolving 2 per cent of Merck's powdered agar in distilled water at about 80°C The impurities in both instances were removed by sedimentation as follows —After melting, the whole mass was put in a separating funnel with the stop-cock closed, and kept in an autoclave for a few hours at 80°C When all the impurities have settled down, the funnel is removed from the autoclave, and the stop-cock opened to allow the sedimented impurities to run off When they have been removed the stop-cock is closed and the contents stored in a flask under sterile conditions

(*b*) Electrolytes used —The following electrolytes were tested with regard to their effects in retarding or accelerating the diffusion of the alkaloid —

Sodium carbonate, sodium bicarbonate, sodium hydroxide, hydrochloric acid, sulphuric acid, nitric acid, formic acid, acetic acid, propionic acid, oxalic acid, malonic acid, succinic acid, citric acid, sodium nitrate, potassium nitrate, barium nitrate, glucose, sodium phosphate and sodium citrate

These chemicals were used in N/10 molar solutions and prepared from Merck's pure products in twice distilled water from a resistant glass Cotton plugs were used in place of cork or rubber stoppers in the distilling apparatus The first and the last parts of the distillate were discarded

(*c*) Cinchona alkaloid—The salts selected for this investigation were the bihydrochloride and the bisulphate of quinine, both of which are commonly used in the treatment of malaria The quinine solution consisted of 5 per cent of the Burroughs Wellcome's pure brand

(*d*) Method of test—To test the diffusion of quinine through the colloidal systems in the presence of various electrolytes test tubes of equal bore were taken and 10 c c of the melted gel was poured in each tube, and then 1 c c of the electrolyte was added, while it was still warm, the contents being well mixed by shaking Silver nitrate or barium chloride were then added in the proportion of 1 c c of a 5 per cent aqueous solution to the still fluid contents of each of the tube to act as indicators for the detection of the diffusion band of chloride or sulphate of quinine respectively When the gel had set, 1 c c of 5 per cent aqueous solution of the alkaloid was poured at the top The depth of the diffusion band was measured in centimetres by means of a travelling microscope after varying periods of time

2 *Results of experiments*

TABLE I (*a*)

Penetration of quinine bihydrochloride in agar-agar and gelatin gels —

Each tube containing —

Gel—10 c c , 5 per cent $AgNO_3$—1 c c , N/10 Electrolytes—1 c c , 5 per cent quinine bihydrochloride—1 c c

ELECTROLYTES USED	NaOH cm		Na_2CO_3 cm		$NaHCO_3$ cm		*NORMAL, WITHOUT ANY ELECTROLYTES cm	
Gels	Agar	Gelatin	Agar	Gelatin	Agar	Gelatin	Agar	Gelatin
Time in hours								
3	0·7	0·9	0·6	0·8	0·5	0·7	0·4	0·6
6	1·1	1·3	0·8	1·1	0·7	0·9	0·6	0·9
9	1·3	1·6	1·2	1·5	1·0	1·2	0·8	1·2
15	1·6	2·3	1·5	2·0	1·3	1·6	1·0	1·5
24	2·0	2·9	1·8	2·5	1·6	2·1	1·2	1·9
39	2·5	3·0	2·3	3·0	2·0	2·6	1·4	2·3
60	3·0	3·8	2·8	3·4	2·5	3·0	1·6	2·6

*Similar controls were used in all the experiments and as the results were the same, the figures have not been repeated in the other tables

TABLE I (*b*)

Penetration of quinine bisulphate

Each tube containing —

Gel—10 c c , 5 per cent $BaCl_2$—1 c c , N/10 Electrolytes—1 c c , 5 per cent quinine bisulphate—1 c c

ELECTROLYTES USED	NaOH cm		Na_2CO_3 cm		$NaHCO_3$ cm		*NORMAL, WITHOUT ANY ELECTROLYTES cm	
Gels	Agar	Gelatin	Agar	Gelatin	Agar	Gelatin	Agar	Gelatin
Time in hours								
3	0·5	0·6	0·4	0·5	0·3	0·4	0·3	0·4
6	0·7	0·8	0·6	0·7	0·5	0·6	0·4	0·5
9	1·0	1·2	0·9	1·0	0·7	0·9	0·5	0·6
15	1·4	1·6	1·2	1·3	0·9	1·2	0·7	0·8
24	1·7	1·9	1·5	1·7	1·3	1·5	0·8	1·0
39	2·0	2·2	1·8	2·0	1·6	1·8	0·9	1·1
60	2·2	2·7	2·0	2·3	1·8	2·0	1·0	1·2

*Similar controls were used in all the experiments and as the results were the same, the figures have not been repeated in the other tables

TABLE II (*a*)

Penetration of quinine bisulphate in agar-agar and gelatin gels —

Each tube containing —

Gel—10 c c , 5 per cent $AgNO_3$—1 c c , N/10 Electrolytes—1 c c , 5 per cent quinine bihydrochloride—1 c c

ELECTROLYTES USED	HCl cm		HNO_3 cm		H_2SO_4 cm	
Gels	Agar	Gelatin	Agar	Gelatin	Agar	Gelatin
Time in hours						
3	0·7	0·9	0·6	0·8	0·5	0·7
6	1·0	1·3	0·9	1·2	0·7	1·0
9	1·3	1·5	1·2	1·4	1·0	1·2
15	1·6	2·1	1·5	1·9	1·3	1·6
24	1·9	2·7	1·8	2·5	1·6	2·0
39	2·4	3·1	2·1	2·9	1·9	2·4
60	2·9	3·6	2·5	3·3	2·2	2·8

TABLE II (*b*)

Penetration of quinine bisulphate in agar-agar and gelatin gels —
Each tube containing —
Gel—10 c c , 5 per cent $AgNO_3$—1 c c , N/10 Electrolytes—1 c c , 5 per cent quinine bisulphate—1 c c

ELECTROLYTES USED	HCl cm		HNO_3 cm		H_2SO_4 cm	
Gels	Agar	Gelatin	Agar	Gelatin	Agar	Gelatin
Time in hours						
3	0·5	0·6	0·4	0·5	0·3	0·4
6	0·7	0·8	0·6	0·7	0·5	0·6
9	1·0	1·2	0·9	1·0	0·7	0·9
15	1·3	1·6	1·2	1·3	0·9	1·2
24	1·6	1·9	1·4	1·5	1·1	1·4
39	1·9	2·2	1·6	1·8	1·3	1·7
60	2·1	2·5	1·8	2·1	1·5	1·9

TABLE III (*a*)

Penetration of quinine bihydrochloride in agar-agar and gelatin gels —
Each tube containing —
Gel—10 c c , 5 per cent $AgNO_3$—1 c c , N/10 Electrolytes—1 c c , 5 per cent quinine bihydrochloride—1 c c

ELECTROLYTES USED	$NaNO_3$ cm		KNO_3 cm		$Ba(NO_3)_2$ cm	
Gels	Agar	Gelatin	Agar	Gelatin	Agar	Gelatin
Time in hours						
3	0·5	0·7	0·4	0·7	0·4	0·6
6	0·7	0·9	0·6	0·9	0·6	0·8
15	1·1	1·6	1·0	1·7	1·0	1·5
24	1·3	1·9	1·2	2·0	1·2	1·8
39	1·5	2·3	1·4	2·4	1·4	2·3
60	1·7	2·6	1·6	2·7	1·6	2·6

TABLE III (*b*)

Penetration of quinine bisulphate in agar-agar and gelatin gels —
Each tube containing —
Gel—10 c c , 5 per cent $AgNO_3$—1 c c , N/10 Electrolytes—1 c c , 5 per cent quinine bisulphate—1 c c

ELECTROLYTES USED	$NaNO_3$ cm		KNO_3 cm		Ba (NO_3) cm	
Gels	Agar	Gelatin	Agar	Gelatin	Agar	Gelatin
Time in hours						
3	0·3	0·4	0·3	0·4	0·3	0·4
6	0·4	0·5	0·4	0·5	0·4	0·5
9	0·5	0·6	0·5	0·7	0·5	0·7
15	0·7	0·8	0·6	0·9	0·6	0·8
24	0·9	1·0	0·8	1·1	0·8	1·0
39	1·0	1·1	0·9	1·3	0·9	1·1
60	1·1	1·2	1·0	1·1	1·0	1·2

TABLE IV (*a*)

Penetration of quinine bihydrochloride in agar-agar and gelatin gels —
Each tube containing —
Gel—10 c c , 5 per cent $AgNO_3$—1 c c , N/10 Electrolytes—1 c c , 5 per cent quinine bihydrochloride—1 c c

ELECTROLYTES USED	FORMIC ACID cm		ACETIC ACID cm		PROPIONIC ACID cm	
Gels	Agar	Gelatin	Agar	Gelatin	Agar	Gelatin
Time in hours						
3	0·5	0·7	0·4	0·6	0·3	0·5
6	0·7	0·9	0·6	0·8	0·5	0·7
9	0·9	1·2	0·8	1·1	0·6	0·8
15	1·1	1·5	1·0	1·4	0·8	1·1
24	1·5	2·0	1·2	1·8	1·0	1·4
39	1·8	2·5	1·4	2·2	1·2	1·8
60	2·1	2·9	1·6	2·6	1·4	2·2

TABLE IV (*b*)

Penetration of quinine bisulphate in agar-agar and gelatin gels — Each tube containing —

Gel—10 c c , 5 per cent $AgNO_3$—1 c c , N/10 Electrolytes—1 c c , 5 per cent quinine bisulphate—1 c c

ELECTROLYTES USED	FORMIC ACID cm		ACETIC ACID cm		PROPIONIC ACID cm	
Gels	Agar	Gelatin	Agar	Gelatin	Agar	Gelatin
Time in hours						
3	0·4	0·5	0·3	0·4	0·2	0·3
6	0·5	0·7	0·4	0·5	0·3	0·4
9	0·7	0·9	0·6	0·7	0·4	0·5
15	0·9	1·1	0·7	0·9	0·5	0·6
24	1·1	1·4	0·9	1·1	0·7	0·8
39	1·3	1·8	1·1	1·2	0·8	1·0
60	1·5	2·0	1·1	1·3	0·9	1·1

TABLE V (*a*)

Penetration of quinine bihydrochloride in agar-agar and gelatin gels — Each tube containing —

Gel—10 c c , 5 per cent $AgNO_3$—1 c c , N/10 Electrolytes—1 c c , 5 per cent quinine bihydrochloride—1 c c

ELECTROLYTES USED	OXALIC ACID cm		MALONIC ACID cm		SUCCINIC ACID cm		CITRIC ACID cm	
Gels	Agar	Gelatin	Agar	Gelatin	Agar	Gelatin	Agar	Gelatin
Time in hours								
3	0·6	0·8	0·5	0·7	0·4	0·6	0·7	0·9
6	0·8	1·1	0·7	0·9	0·6	0·8	1·0	1·2
9	1·2	1·4	0·9	1·2	0·8	1·2	1·4	1·7
15	1·5	1·8	1·2	1·7	1·1	1·5	1·7	2·2
24	1·9	2·3	1·5	2·2	1·3	2·0	2·0	2·6
39	2·1	2·7	1·8	2·6	1·5	2·4	2·5	3·0
60	2·3	3·0	2·0	2·8	1·7	2·6	2·8	3·4

TABLE V (*b*)

Penetration of quinine bisulphate in agar-agar and gelatin gels — Each tube containing —

Gel—10 c c , 5 per cent $AgNO_3$—1 c c , N/10 Electrolytes—1 c c , 5 per cent quinine bisulphate—1 c c

ELECTROLYTES USED	OXALIC ACID cm		MALONIC ACID cm		SUCCINIC ACID cm		CITRIC ACID cm	
Gels	Agar	Gelatin	Agar	Gelatin	Agar	Gelatin	Agar	Gelatin
Time in hours								
3	0·5	0·6	0·4	0·5	0·3	0·4	0·6	0·7
6	0·7	0·8	0·6	0·7	0·4	0·5	0·8	0·9
9	0·9	1·1	0·8	0·9	0·5	0·6	1·0	1·2
15	1·1	1·3	1·0	1·2	0·7	0·8	1·3	1·5
24	1·3	1·6	1·2	1·5	0·8	1·0	1·5	1·8
39	1·5	1·9	1·4	1·8	1·0	1·2	1·7	2·1
60	1·8	2·0	1·6	1·9	1·2	1·3	1·9	2·5

TABLE VI (*a*)

Penetration of quinine bisulphate in agar-agar and gelatin gels — Each tube containing —

Gel—10 c c , 5 per cent $AgNO_3$—1 c c , N/10 Electrolytes—1 c c , 5 per cent quinine bihydrochloride—1 c c

ELECTROLYTES USED	SOD CITRATE cm		SOD PHOSPHATE cm		GLUCOSE cm	
Gels	Agar	Gelatin	Agar	Gelatin	Agar	Gelatin
Time in hours						
3	0·6	0·9	0·5	0·7	0·4	0·6
6	0·9	1·3	0·7	1·0	0·6	0·8
9	1·2	1·7	0·9	1·5	0·7	1·1
15	1·5	2·2	1·2	2·1	0·9	1·3
24	1·9	2·7	1·4	2·4	1·2	1·7
39	2·3	3·1	1·6	2·9	1·6	2·1
60	2·7	3·5	1·8	3·0	1·8	2·6

TABLE VI (*b*)

Penetration of quinine bisulphate in agar-agar and gelatin gels —
Each tube containing —
Gel—10 c c , 5 per cent $AgNO_3$—1 c c , N/10 Electrolytes—1 c c , 5 per cent quinine bisulphate—1 c c

ELECTROLYTES USED	SOD CITRATE cm		SOD PHOSPHATE cm		GLUCOSE cm	
Gels	Agar	Gelatin	Agar	Gelatin	Agar	Gelatin
Time in hours						
3	0·5	0·6	0·5	0·6	0·4	0·5
6	0·7	0·9	0·7	0·9	0·5	0·6
9	0·9	1·2	0·9	1·1	0·6	0·7
15	1·1	1·5	1·0	1·3	0·7	0·9
24	1·3	1·9	1·2	1·7	0·9	1·2
39	1·5	2·2	1·4	2·0	1·0	1·4
60	1·7	2·5	1·6	2·3	1·1	1·6

3 *Discussion of results*

The experiments tabulated above clearly show that the penetration of quinine bihydrochloride and quinine bisulphate through a colloidal system such as gelatin and agar-agar gels is markedly influenced by the presence of certain electrolytes, the extent of this penetration being dependent on their chemical nature It was interesting to find that in every case the penetration of alkaloid was quicker in gelatin gels than in agar, and also that quinine bihydrochloride diffuses more than quinine bisulphate

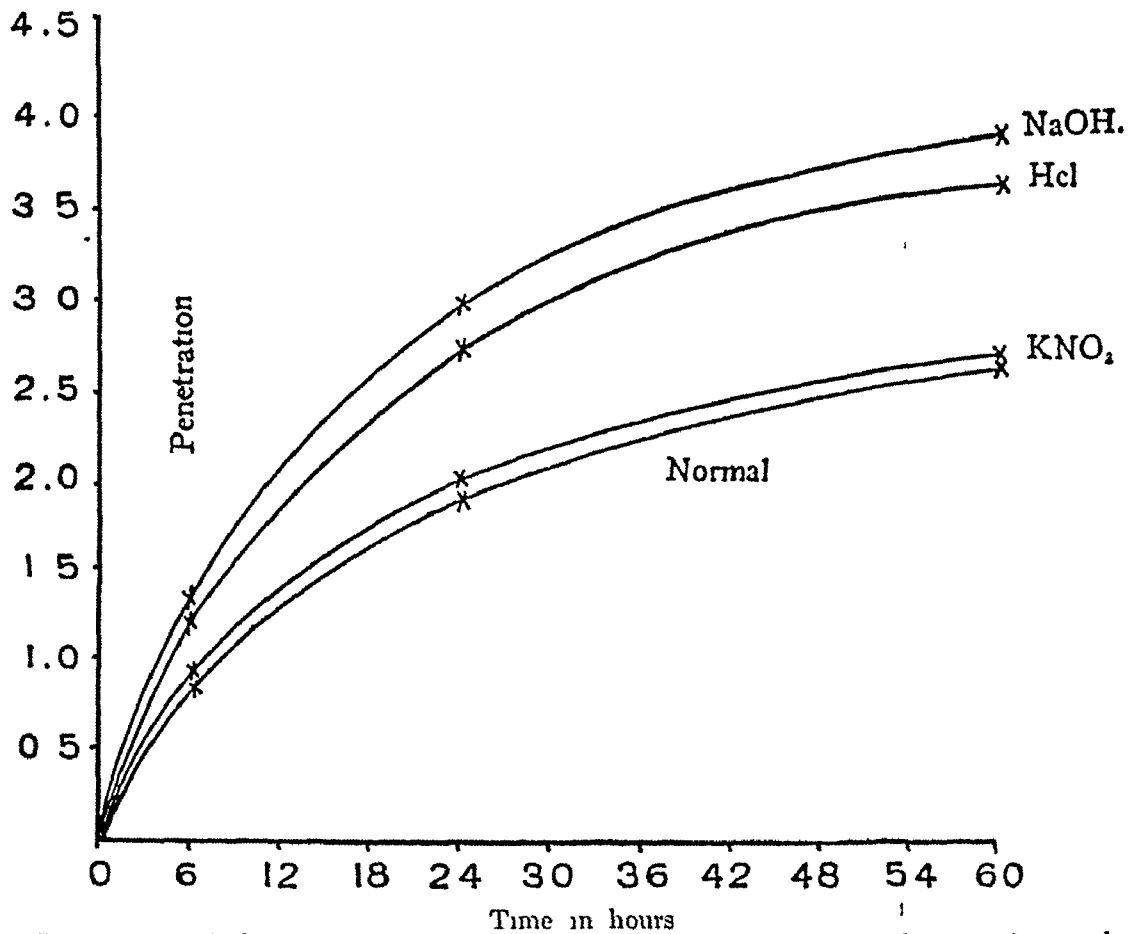
(*a*) Penetration affected by mineral acids, alkalies and neutral salts

If we take each set of electrolytes separately we find, as seen from Tables I (*a*) and I (*b*), that all the three alkalies used increase the penetration of the alkaloid into the gel body, the order followed being $NaOH > Na_2CO_3 > NaHCO_3$ It appears that the stronger the alkali, the greater is the diffusion of the alkaloid

Tables II (*a*) and II (*b*) indicate that the three mineral acids, i e , nitric, hydrochloric and sulphuric, accelerate the diffusion of the alkaloid in the colloidal system in the following order —$HCl > HNO_3 > H_2SO_4$, which resembles that observed in the case of alkalies, namely, the stronger the acid, the more does it help the diffusion of the alkaloid

The presence of neutral salts, i e , sodium nitrate, potassium nitrate, and barium nitrate does not seem to have any effect on the penetration of the alkaloid A slight variation from the normal penetration range is, however, observed in Tables III (*a*) and III (*b*) but this may possibly be due to experimental error

TEXT-FIG 1



Penetration of the three types of electrolytes giving maximum results in gelatin gels

From the study of Tables I, II and III it appears that whereas the presence of mineral acids and alkalies in the colloidal systems promotes the diffusion of the alkaloid, the neutral salts are practically without any effect This behaviour of the three types of electrolytes can be well represented by curves as shown below (Text-figs 1 and 2)

(*b*) Penetration affected by organic acids

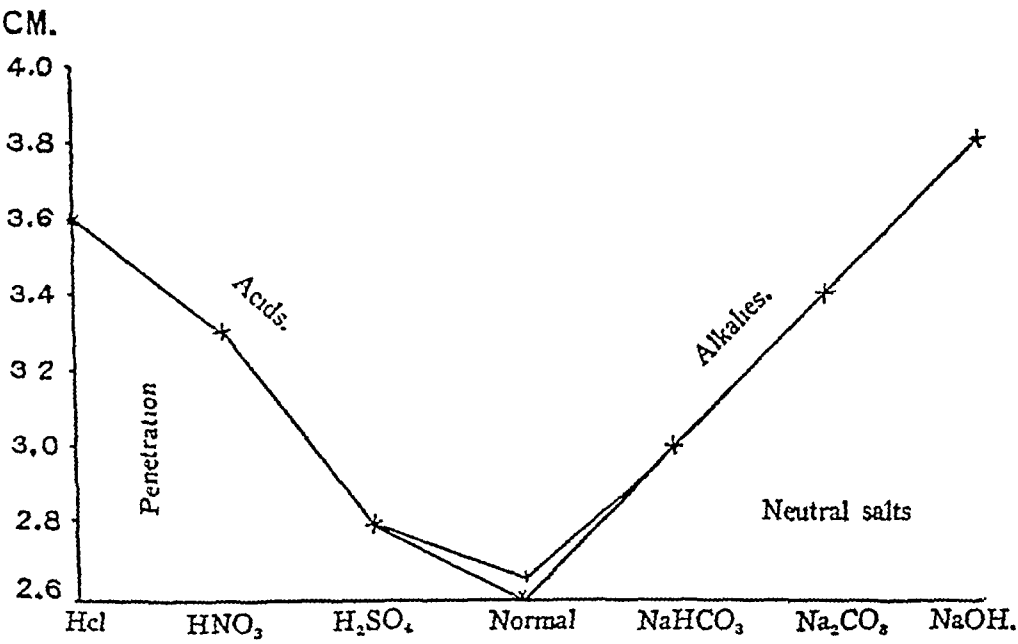
Tables IV (*a*) and IV (*b*) show that in the case of monobasic acids, increased penetration of the alkaloid above the normal range, was only recorded with the lowest member of the series, i e , formic acid, while acetic acid has no effect and propionic acid, the highest member of the three used, retards the diffusion to some extent This holds good both with quinine bihydrochloride and quinine bisulphate

It appears as if the power to allow the penetration of the alkaloid is somehow related to the molecular weight* of the acids used, because the lower the molecular weight of the member of the homologous series the higher is the extent of penetration and vice versa

It is clear from Tables V (*a*) and V (*b*) that all the three dibasic acids† used, accelerate the penetration of the alkaloid in the colloidal systems, and

TEXT-FIG 2

The amount of penetration affected by the presence of each electrolyte in relation to one another within the three types studied is shown as



the same relationship of the degree of penetration was observed as in the case of monobasic acids

The polybasic acid, i e, citric acid, allows the greatest amount of penetration of the alkaloid as compared to the monobasic and dibasic acids

The data obtained by using the above mono-, di-, and polybasic acids as electrolytes is very interesting It indicates that the monobasic acids allow least penetration of the alkaloid, while the polybasic acids help it most, and also that the lowest members of the series allow more penetration than their higher homologues

---

* The molecular weights of the group of acids studied are as given below —

Formic acid	46 03
Acetic acid	60·04
Propionic acid	74 07

† The molecular weights of the three acids tried are —

Oxalic acid (anhyd)	90·06
Malonic acid	104 05
Succinic acid	118·07

(*d*) Method of measurement

The *Chara* cells, ten in number, were placed in the groove of the conductivity cell, and the upper block fitted on the lower one as shown in Plate XXII, fig 2* The resistance was measured in ohms by a Slide-Wire Wheatstone Bridge fitted with sensitive telephone, tuned to a frequency During the electrolysis of an aqueous solution between platinum electrodes, gases are evolved, and a back electromotive force (polarization e m f) is produced Under such conditions it is not readily possible to measure the resistance of a liquid conductor by means of a direct current, and an alternating current, as is given by an induction coil, was therefore employed The induction coil should be placed at a distance of about three feet from the telephone so that it does not directly affect it

The experiments were performed in a constant-temperature room at about 19 5°C to avoid discrepancies due to variations in temperature

(*c*) Calculations

The results were calculated by the formula given below —

If R is the known resistance inserted in the box, and if the sliding contact at the position of minimum sound in the telephone divides the bridge wire in the ratio x 100-x, where x is the bridge reading in centimetres, then the resistance V, of the solution under test is given by $V=\frac{R\ (100-X)}{X}$ hence the conductivity of the solution is $1/V=\frac{X}{R\ (100-X)}$.

(3) *Results of experiments*

TABLE VII

Resistance and conductivity of the solution (R = 9,000)

Electrolytes used	Na_2CO_3		$NaHCO_3$		Citric Acid	
	Resistance	Conductivity	Resistance	Conductivity	Resistance	Conductivity
Time in minutes						
10	2,250	$0\cdot444\times10^{-3}$	3,500	$0\ 286\times10^{-3}$	2,842	$0\cdot351\times10^{-3}$
20	3,000	$0\cdot333\times10^{-3}$	3,855	$0\ 259\times10^{-3}$	3,162	$0\ 315\times10^{-3}$
30	3,327	$0\cdot300\times10^{-3}$	4,235	$0\ 235\times10^{-3}$	3,500	$0\ 286\times10^{-3}$
40	3,594	$0\ 278\times10^{-3}$	4,636	$0\cdot215\times10^{-3}$	3,855	$0\cdot259\times10^{-3}$
50	3,857	$0\cdot258\times10^{-3}$	5,285	$0\ 189\times10^{-3}$	4,235	$0\cdot235\times10^{-3}$
60	Constant	Constant	Constant	Constant	4,636	$0\cdot215\times10^{-3}$
80					5,282	$0\ 189\times10^{-3}$

*The upper block with attached electrodes is always kept in distilled water when not in use, to keep the electrodes wet and avoid the possibility of the formation of bubbles when introduced into the electrolyte solution because if some bubbles remain sticking, a remarkable difference in the results will be observed

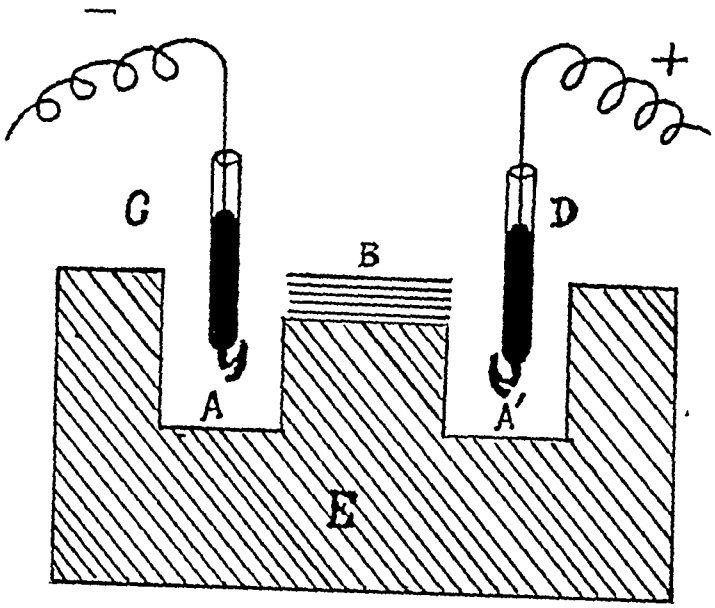


Fig 1 Cross section of the conductivity cell (lower half only) A and A¹, depressions in the paraffin block E C and D, electrodes containing mercury B groove for placing the *Chara* cells

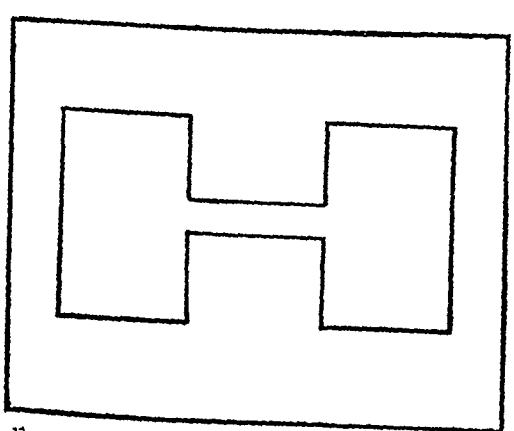


Fig 3 Dorsal view of the lower block of the cell

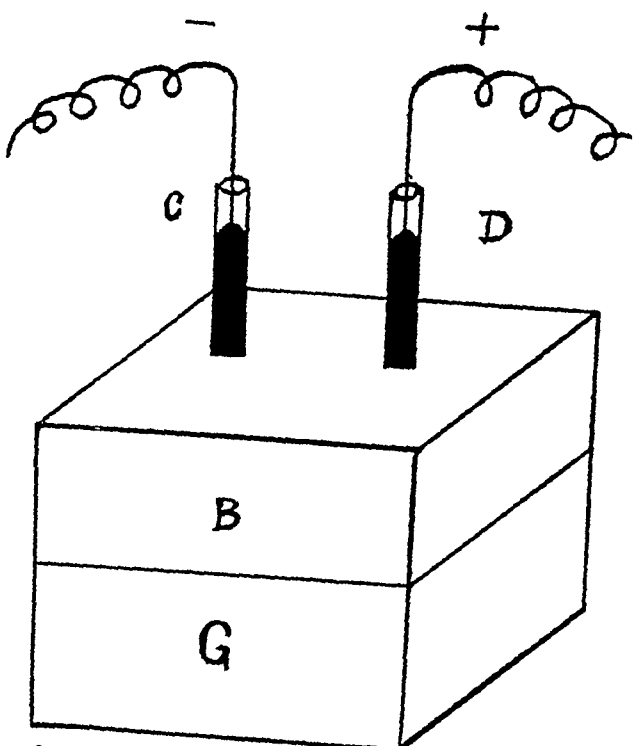


Fig 2 Conductivity cell C and D, mercury electrodes B and G, upper and lower paraffin blocks

TABLE VIII

Resistance and conductivity of the solution (R = 9,000)

Electrolytes used	KNO_3		$NaNO_3$		Normal without any electrolyte	
	Resistance	E Conductivity	Resistance	E Conductivity	Resistance	Conductivity
Time in minutes						
10	9,750	0.102×10^{-3}	10,556	0.91×10^{-4}	12,428	0.81×10^{-4}
20	11,000	0.91×10^{-4}	11,151	0.87×10^{-4}	12,951	0.77×10^{-4}
30	12,128	0.81×10^{-4}	12,128	0.81×10^{-4}	13,500	0.74×10^{-4}
10	13,500	0.71×10^{-4}	13,500	0.71×10^{-4}	14,076	0.71×10^{-4}
50	14,684	0.68×10^{-4}	14,684	0.68×10^{-4}	14,684	0.68×10^{-4}
60	Constant	Constant	Constant	Constant	15,324	0.65×10^{-4}
80	Constant	Constant	Constant	Constant	Constant	Constant

(4) *Discussion of results*

When an electrolyte was added to the dilute quinine solution in the conductivity cell, it was noticed that the electrical conductivity greatly increased, depending upon the electrolyte used. After intercepting the *Chara* cells it was observed, as is shown by the tables, that the electrical conductivity of the electrolyte decreased and resistance increased with time, whereas in the case of the *Chara* cells the conductivity increased and resistance decreased. This indicates that a proportionate amount of the quinine is probably permeating into the plasma membrane of the *Chara* cells. This fact was confirmed by chemical methods. The *Chara* cells were gently removed from the groove of the conductivity cell and washed well with running tap water followed by distilled water, and then carefully dried on a filter paper. This was done to remove traces of quinine adhering to the surface of the cell. They were then placed on a clean glass slide, and one end cut with a safety razor blade. With the help of a slight pressure applied to the cells, a clear cell sap flows out. A drop of Tamet's Reagent* was then added, which produced a distinct opacity. Further the cell sap when diluted with water containing sulphuric acid showed fluorescence. The cell sap obtained from the normal cells did not give either of these tests. The above tests were quite enough to prove that the cell sap contained quinine alkaloid.

The preceding tables indicate that whereas sodium carbonate, sodium bicarbonate, formic acid, and citric acid accelerate the permeability of the

* Dissolve 1.35 grms. of mercuric perchloride in 75 c.c. water and 5 grms. potassium iodide in 20 c.c. water in a 100 c.c. graduated flask. Pour mercuric solution into the iodide solution under agitation and fill up to mark with water (Nierenstein, 1919).

quinine salt in the cell, the neutral salts, i e, sodium nitrate and potassium nitrate are practically without any effect Calcium chloride seemed to have an antagonistic effect with regard to the permeability of the alkaloid The electrical conductivity of the cells did not rise, nor did the cell sap show the presence of the alkaloid by any of the tests mentioned above

Experiments were also conducted with the mineral acids and sodium hydroxide, but the concentration used proved toxic to *Chara* cells which died during the course of the experiment

III Comparison of the results obtained by penetration and permeability experiments

The experimental data obtained with electrical conductivity show results which appear parallel to those found in the diffusion through gels It is of great interest to observe that electrolytes which promote the acceleration of the diffusion of quinine in the colloidal system also increase the permeability of the *Chara* cells to this alkaloid

It has thus been shown that penetration of quinine salts through colloidal systems such as gelatin gels and agar-agar gels is markedly influenced by the presence of certain electrolytes Further the exact modification in this process is dependent upon the chemical nature of the salts

The degree of diffusion is closely related to the systematic position of the electrolytes in the homologous series

The chief electrolytes present in blood, e g, sodium phosphate, sodium carbonate, sodium bicarbonate, sodium chloride, etc, have been found to materially affect the permeability of the colloidal systems tested This finding seems to be related to the discovery by Acton and Chopra (1923) that certain chemical and physical changes affecting the cell surfaces increase or inhibit the action of many drugs

The results obtained in the present investigation suggest that the effect of certain electrolytes on the penetration and permeability of cells by quinine is possibly one of the factors responsible for the beneficial effects of quinine and alkali treatment of malaria advocated by Sinton (1923)

A possible explanation of these results is that the reactivity of quinine is greater on account of the greater and quicker penetration of the alkaloid in cell bodies under these conditions The results on diffusion show clearly that the chemical analogue of the living cell is considerably affected with respect to this property in acid and in alkaline substrate as compared with the neutral one

Summary

1 A modification of Osterhout's electrical conductivity cell is described, which eliminates various disturbing factors

2 Penetration —The effect of the presence of various electrolytes on the penetration of quinine salts has been studied quantitatively on the chemical

analogues of living cells It is shown that the penetration of quinine salts through colloidal systems of gelatin and agar-agar gels is considerably influenced in acid and alkaline media, while the presence of neutral salts has practically no effect The stronger acids and alkalies allow more penetration than the weaker ones The degree of penetration of the electrolytes is closely related to their chemical nature and systematic position in the homologous series

3 Permeability —Electrical conductivity has been taken as a measure of permeability of quinine salts into the living protoplasm of *Chara* cells It was observed that permeability of quinine salts was markedly affected by alkalies and that the neutral salts did not produce any change The mineral acids in the concentration used had a toxic effect and the cells died after a short time

4 It was interesting to see that the results obtained with penetration of quinine salts into the colloidal systems and permeability into the living protoplasm of vegetable cells are nearly parallel

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FURTHER OBSERVATIONS ON THE MORPHOLOGY OF *PLASMODIUM FALCIPARUM* WITH SPECIAL REFERENCE TO THE FINDINGS IN TWO FATAL CASES OF MALARIA *

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THIS memoir is made up of two separate topics The first, 'A', refers to the fate of the merozoites of *Plasmodium falciparum* in mixed cultures, and the second, 'B', to its morphology in fatal cases of septicæmic malaria

A FATE OF THE MEROZOITES OF *Plasmodium falciparum* IN MIXED CULTURES

In describing the fate of the merozoites in culture, it has been pointed out elsewhere (Row, 1929), that under the usual conditions of malarial infections, most of the merozoites are destroyed either by plasmolysis or by phagocytosis, and that only such of them as had escaped this destruction are responsible for the succeeding paroxysm—until the infection is either completely spent or it culminates in the initiation and advent of the crescents—an ideal state of equilibrium between the host and the parasite

It may be permissible here to add one more experimental observation to those already published, which made it possible to follow the parasite's accelerated effort towards crescent formation in my attempts at sub-cultures under conditions where the baneful influences of both the serum and the phagocytes on the parent cultures were reduced to a minimum The accelerated development was brought about by using a mixture of *Plasmodium vivax* and *Plasmodium falciparum* for culture, as by this manipulation it is found that the *Plasmodium falciparum* supplants the *Plasmodium vivax* and the preliminary changes in the culture medium during the growth and subsequent destruction

* This work was done under the auspices and with the aid of a grant from the Indian Research Fund Association (1929)

of these parasites, introduce conditions favourable to a hastened production of such schizonts of the *Plasmodium falciparum* as are capable of yielding but two or even one merozoite—a stage which indicates the inauguration of the gametocytes as suggested by the previous observations Could these mixed infections be one of the factors responsible for the presence of the crescents in carriers in endemic areas ?

The Experiment —A mixture of *Plasmodium vivax* and *Plasmodium falciparum* rings contained in red blood cells from two separate patients was planted in glucosed ascitic fluid and when the *Plasmodium falciparum* schizonts had fully matured with the full load of merozoites, sub-cultures were made from the deposit in a suspension of fresh red blood cells in ascitic fluid and the whole mixture was filtered through cotton-wool so as to ensure (*a*) the elimination of the leucocytes and (*b*) a thorough dilution of the medium derived from the parent culture and of the fresh defibrinated blood used for the sub-culture This sub-culture revealed that the freshly introduced red blood cells were infected with rings inside them and these developed into schizonts in 48 and 72 hours—but into schizonts yielding only one to two and at most only four merozoites—but further sub-cultures carried out on similar lines always failed for want of capacity of these merozoites to infect fresh red blood cells These facts are depicted in Plate XXIII

B The morphology of the merozoites in fatal septicæmic malaria

The fate of the parasite under consideration where the host-parasite association was such that the parasites had all their own way, was not possible of demonstration until material for observation was available at the autopsies of cases dying of a severe malarial infection, which from the microscopic findings of the brain section alone may have been mistaken for the so-called 'Cerebral Malaria,' but which turned out to be, from the examination of other organs, septicæmic malaria Besides no cerebral symptoms or signs were obvious clinically

The clinical and post-mortem notes of these two cases are as follows —

Case No 1 —Male aged 30, was brought into the hospital by the police, who picked him up at the roadside in collapsed condition The admitting officer's notes —

'The patient states that he had an attack of asthma for 15 days and pain in the joints of the lower limbs Temperature 98°F , pulse 90, rhonchi in both lungs, patient dyspnœic and looks ill' On admission into the wards the patient was conscious but too weak to answer questions, breathing hurried and noisy He collapsed and was unable to give any history Heart sounds weak, pulse not felt at wrists, liver and spleen not felt, the patient died half an hour after admission at 11-25 P M

Post-mortem 12 hours after death—Notes —(P M 93 of 1929)

Larynx, trachea, lungs and pleura —Nothing abnormal

Heart —Smaller than normal with a thick layer of fat on the surface

Heart muscle paler than normal No valvular lesions detected

Pericardium—Nothing abnormal

Aorta—Showed advanced atheromatous condition in the proximal (ascending) and transverse parts of the arch A few patches of early atheromatous condition are found in the descending thoracic and abdominal parts

Coronaries—Normal

Kidneys—Smaller and paler than normal Capsule slightly adherent to the renal surface There is fairly good amount of fat in the pelvis Cortex and medulla have normal relations to each other

Ureter and Bladder—Nothing abnormal

Liver—Normal in size of dark slate colour, on section it was found to be hyperæmic

Gall-Bladder and Bile Ducts—Nothing abnormal

Spleen—Enlarged to about one and a half its normal size On section it was found to be dark, pulp was soft The dark slate colour of liver and spleen was remarkable in contrast to the pallor of the heart muscle and kidneys The smears from the splenic pulp showed malignant tertian parasites in large numbers

Tongue, pharynx and œsophagus—Nothing abnormal

Stomach—Mucous membrane rugose and uniformly congested contained yellowish semi-fluid material

Intestines—Nothing abnormal

Brain—Nothing abnormal detected macroscopically

Case No 2—Male aged 20, was admitted for fever of one and a half months' duration on 7th December, 1929 No temperature on admission, but intensely pale and breathless, clinical examination revealed cardiac weakness, loud hæmic murmur in the pulmonary area œdema of ankles and face Blood examination—Hydræmic, no parasites found Differential count—Polymorphs, 66 per cent, Lymphocytes, 24 per cent, Hyalines, 10 per cent, Eosinophiles, 0 per cent On the fourth day of admission a few M T rings were found in finger blood, but no rise of temperature since the day of admission, until the afternoon, when after a rigor the temperature shot up to 105°F, with respiratory distress, with shallow and fast breathing, when seen at 5 P M the patient was conscious, temperature 104°F and tending to go down, but the condition of the patient was low and dyspnœa marked, the patient died at 6-30 P M

Post-mortem 18 hours after death

Post-mortem Notes—(P M No 98 of 1929)

Lungs—Both lungs are pale and crepitant under fingers On section froth and small amount of blood exuded on pressure

Heart—Smaller and muscle paler than normal No valvular lesions detected Smears from heart blood showed malignant and benign tertian parasites

Aorta—Pale and smooth

Kidneys—Normal in size, very pale on section, capsule strips easily, cortex and medulla normal

Ureters and Bladder—Nothing abnormal

Liver—Dark slate coloured and hyperæmic

Spleen—Dark slate coloured, pulp soft Smears from splenic pulp showed benign and malignant tertian parasites

Stomach—Nothing abnormal

Intestines—Nothing abnormal

Brain—Paler than normal

Laboratory findings—Heart blood smears show a very rich infection of red blood cells some of these contain two or three rings of *Plasmodium falciparum* (Plate XXIII, fig 2) Smears from spleen as stated above showed the parasites in all stages of development, rings, merozoites, gametocytes and gametes, being all detected in the same specimen in abundance and the pigment laden leucocytes and endothelial cells (Plate XXIII, fig 3) Smears from the liver were remarkable for the absence of demonstrable parasite but richness in hæmozoin deposit both inside and outside the large endothelial cells The sections of the brain, the heart muscle, the pancreas, the suprarenals, and the kidneys reveal identical conditions in the richness of the parasites and in their stage of development in every situation wherever the capillaries were found full of stagnant blood These are best seen in the microphotographs (Plate XXIV, figs 4 to 8), and require very little description, beyond stating that the identity of the parasite was made out by the condensed accumulation of the hæmozoin in the form of a dense brown black dot, the cytoplasm of the parasite being noticed as a thin rim round each dot taking up a blue stain in Giemsa-stained sections and the absence of chromatin in any form indicating its lysis in the cytoplasm

It appears from a study of these histological sections that when the patient was in *articulo mortis* and therefore had lost all powers of defence, the parasites underwent such a rapid and uncontrolled multiplication, that the merozoites overflowed invading almost every red blood cell and were disseminated broadcast, wherever the circulation was able to carry them but with the failure of circulation and attendant stasis, remained in situ in the capillaries of all the organs The only situation where the parasites were not clearly demonstrable was the liver where they were apparently disposed of by the Kupffer cells which were found loaded with massive deposits of hæmozoin, whereas in the regions of the capillaries where the conditions for stasis, conglutination and thrombosis were at the optimum, the parasite made a desperate and last effort resulting in the above described morphological changes, characterized by shrinkage of the cytoplasm, karyolysis of the chromatin and precipitation of the hæmozoin within their bodies

I desire to express my thanks to the members of the staff of the Pathology School, viz, to Dr H S Patel, Dr J L Saldanha, Dr P L Deshmukh,

Dr V B Athavale, and Dr D W Soman, who have co-operated with me in this investigation

REFERENCE

Row R (1929) On some observations on the malarial parasites grown aerobically in simple cultures, etc *Ind Jour Med Res*, XVI, 4 p 1120

EXPLANATION OF PLATE XXIII

Fig 1 Shows the various stages of development of the merozoites in sub-culture from a mixed culture of *P vivax* and *P falciparum*

„ 2 Blood film from the heart blood taken post-mortem of case 2 (1/12th objective, oil immersion)

„ 3 Smear from splenic pulp, case 1 (1/12th objective, oil immersion)

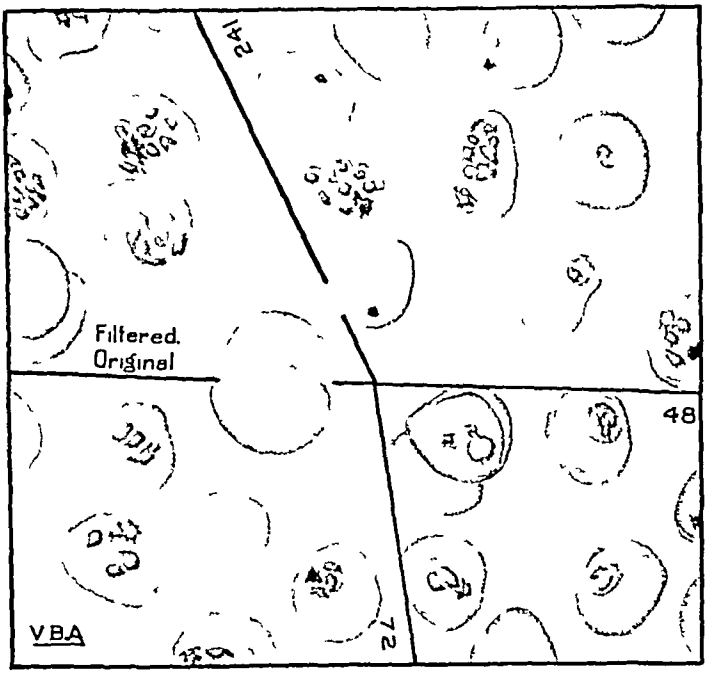

FIG 1

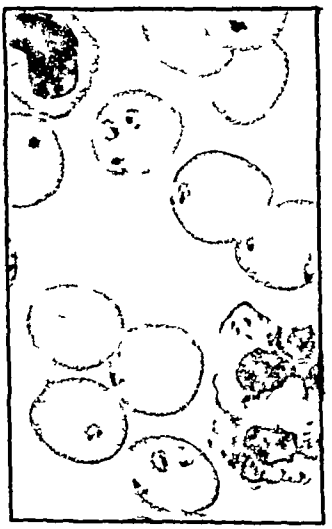

FIG 2

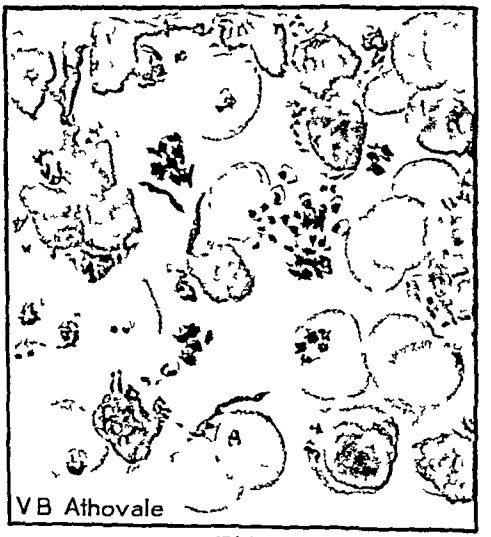

FIG 3

Fig 4

Fig 5

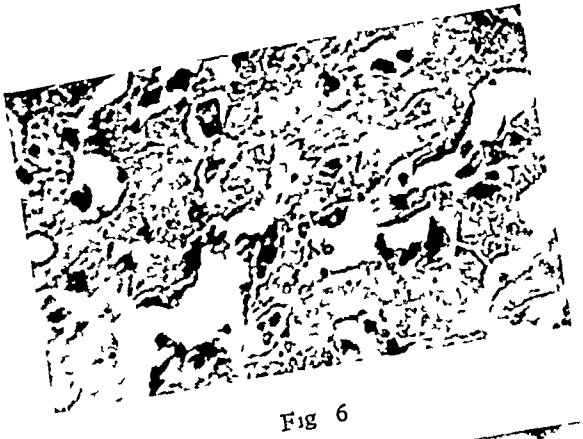

Fig 6

Explanation of Plate XXIV

Fig 4 Microphoto of the section of the cardiac muscle, case 1 Note the separation of the muscle fibres by the capillaries choked with the parasites (1/6th objective)

„ 5 Microphoto of the section of the kidney case 1 (1/6th objective)

„ 6 Microphoto of the section of liver case 1 (1/6th objective)

„ 7 Microphoto of the section of the brain case 1 (1/12th objective, oil immersion)

„ 8 Microphoto of the cardiac muscle of case 1 (1/12th objective, oil immersion)

INTER-RELATIONSHIP OF SOME OF THE IMPORTANT ENDOCRINE GLANDS, WITH SPECIAL REFERENCE TO THE PART THEY PLAY IN INFLUENCING THE COLOUR AND TEXTURE OF THE SKIN

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INTRODUCTION

WHILE investigating the functions of the endocrine glands in certain diseases, some time ago, it was observed by the writer that the action of insulin was different in different cases, that is to say, the rate of fall of blood-sugar, after subcutaneous injection of a proportionate dose of insulin, as well as the reactions resulting therefrom varied widely It was also noticeable at the time that the colour of the individual bore some relationship to the action of insulin

In order to find out whether the colour of animals had anything to do with this variation in the insulin action, the effect of insulin on animals of the same species but of different colour was studied For preliminary investigation, two varieties of the Himalayan rabbit were selected, viz —

(1) The albino variety with pink eyes

(2) The jet-black variety with dark-pigmented eyes

Care was taken in the selection of the particular animals, and a dose of insulin proportionate to the weight of the animals was given, being 1 Physiological Rabbit Unit (P R U)* to each kilogram of the body-weight To avoid irregularities in the result due to the action of food, all rabbits were kept on the same diet and were starved for 16 hours before the test

*One Physiological Rabbit Unit (P R U) is equivalent to 3 clinical units

The result of insulin action on these two types of animals was striking The albino Himalayan type of rabbits were found to be very much more resistant (judged by the lowering of blood-sugar) to the same dose per kilo of insulin than the jet-black variety The jet-black rabbits had an average reduction of 60 per cent of blood-sugar, 2 hours after insulin, while the blood-sugar of the albino type was much *less* reduced after the same dose per kilo Moreover, most of the rabbits of the jet-black variety had reactions following insulin injection, varying from mild to moderate degrees, 25 per cent, having rather severe forms of typical hypoglycæmic reactions One of the most noticeable features of this experiment was that none of the albino type showed the least sign of insulin hypoglycæmia, as a matter of fact, they behaved like normal animals throughout the experiment

Encouraged by the above result, the experiment was tried on the brown Belgian-hare type of rabbits* It was found that this variety of rabbit was also very susceptible to insulin action, even more so than the jet-black variety The average reduction of the blood-sugar, after the same dose of insulin per kilo, was 65 per cent (*see* Table VI) as against 60 per cent of the jet-black variety and 41 6 per cent of the albino variety (*see* Table I) and nearly all rabbits of this type went into hypoglycæmic convulsions, and many had to be revived by intravenous injection of glucose

Having definitely ascertained that the action of insulin varied widely on these three species of rabbits, the next point to consider was whether the colour of the animal had anything to do with this variation in result

The literature on the subject contains a large mass of evidence to show that the adrenals (as well as the pituitary) play an important part in the reproduction of pigments Adrenalin is believed to be closely related to tyrosin, which is a chromogenic substance, that is, it is capable of producing pigments If the adrenals are normally functioning, the tyrosin is converted into adrenalin, but in case of hypo-function of the adrenals, the tyrosin is unable to break down any further and may give rise to pigmented substances

As an example of the theory that the underaction of the adrenal medulla gives rise to increased pigmentation, mention may be made of the classical instance of Addison's disease, which shows all the characteristics of adrenal hypo-function Among other evidences of the association of the colour of

*This variety is used in England, and in the Continent for insulin standardization experiments When insulin came to India, in the latter part of 1922, there was a great deal of controversy among different workers in India, as regards the results of standardization tests as carried on here On a request from Simla, Col H W Acton and the writer worked on this problem for some time and the results of their investigations definitely proved that the alleged deterioration of insulin was more *apparent than real* and was really due to variations existing in the animals experimented upon

Vide (*a*) 'The variability in rabbits used for the assay of insulin' (Bose and Acton, *Ind Med Gaz*, LIX, 7, July, 1924),

(*b*) 'The relationship of the colour of the rabbits to their susceptibility to insulin' (Acton and Bose, *Ind Jour Med Res*, XV, 1, July, 1927)

animals with the function of the medullary substance of the supra-renal glands, may be mentioned that the hair of the Arctic fox turns white during the winter and reverts to a dull brown colour during the summer, a condition correlated to the stress of life that occurs during these seasons in the Arctic Zone

To find out whether the albino Himalayan rabbits would show an increased adrenalin response, as compared to the pigmented rabbits, the following experiment was carried out —

A group of rabbits from each of these two varieties was selected and an uniform dose of adrenalin (0 15 mg per kilo) was given to all, the blood-sugar having been estimated before the injection and one hour after the injection

It was found that while in the case of the albino variety, the blood-sugar, 1 hour after injection of adrenalin, was increased by 132 7 per cent (Table II), that of the Belgian-hare type of rabbits was increased by 36 6 per cent only (Table VII) This difference was very striking

Summarizing the results of the above experiments, it will thus be seen —

(1) That in rabbits of the same species but of different colours, *insulin* causes a much *smaller reduction* in the blood-sugar of the albino variety of rabbits than in either the black or the brown variety,

(2) That in rabbits of the same species but of different colours, *adrenalin* causes a much *higher increase* in the blood-sugar in the albino variety than in the brown type

Experiments on the inter-relationship between the actions of insulin and adrenalin on blood-sugar content

From the foregoing experiments, it will be seen that there is some association between the colour and species of an animal and the output from its adrenal glands In the albino variety of rabbit, we find that the rise of blood-sugar after adrenalin is about 260 per cent more than in the brown variety, whereas the fall of blood-sugar after insulin is markedly less than that of the other variety It seems, therefore, reasonable to suggest, on the basis of evidence which has already been put forward, that the adrenalin content of the albino-Himalayan rabbits is high, and, accordingly, a stimulating dose of adrenalin produces pronounced effect, resulting in a well-marked increase in the blood-sugar The lower rate of fall of blood-sugar after insulin injection in the same rabbits can also be partly explained, as being due to the partial inhibition of the insulin action by the more powerful adrenal action inherent in the animal In a previous experiment done by the writer, it was found that adrenalin antagonizes the action of insulin on blood-sugar, so that when adequate doses of both insulin and adrenalin are given simultaneously, there is neither any rise nor fall of blood-sugar

In the brown Belgian-hare type of rabbits, on the other hand, it would be reasonable to assume that the adrenalin content was low (as evidenced by the poor response to adrenalin injection) and hence insulin had more or less an

unopposed action, lowering the blood-sugar to a much greater extent and producing the symptoms of hypoglycæmia

This experiment also brings home to us a rather valuable deduction, viz —

(1) *That rabbits, which give a well-marked adrenalin response, give a poor insulin response, and vice versa*

(2) *That rabbits, which give a poor adrenalin response, give a violent insulin response*

We have assumed in the above experiments that a marked rise in blood-sugar after a dose of adrenalin is evidence of high adrenal action, and that a poor rise in the blood-sugar denotes a poor adrenal action, the degree of hyperglycæmia produced after a proportionate dose of adrenalin being thus indicative of the adrenal function in a rough and ready way We realize that the above statement is neither complete nor wholly true, because the mechanism by which the amount of sugar in the blood is regulated is a very complicated process We know that there are two groups of ductless glands with antagonistic actions, which control this mechanism, the one group consists of the internal secretion of the pancreas and the parathyroids, the tendency of these being (expressed in simple language) to *check* hyperglycæmia, the other group consists of the thyroid, the suprarenals and the pituitary, the tendency of each of these glands being to mobilize sugar into the blood and *cause* hyperglycæmia The secretions of the former group are influenced by the vagus, and those of the antagonistic group are all controlled by the sympathetic system

That there is some relationship between the action of the suprarenal bodies and the thyroid gland is undoubted, though evidence is not conclusive on the point whether this relationship is a direct one Thyroid, like adrenalin, acts antagonistically to insulin There is plenty of evidence on this point in the literature and it may also be mentioned that small doses of insulin have been found to do much good in cases of Grave's disease The hyperglycæmia, tachycardia, and loss of weight produced by excessive thyroid stimulation, is controlled, to some extent, by the opposing effects of insulin

The adrenals are believed to act through the splanchnic nerve and the hepatic plexus, causing a release of sugar from the glycogen store-house of the liver, thus tending to cause hyperglycæmia It is also believed that the thyroid helps to intensify the action, to a great extent, because it has been shown by Burn and Marks that the liver responds less readily to adrenalin stimulation in the absence of the thyroid

It has already been shown that in the albino Himalayan type of rabbits, the adrenalin response is very high, i e, the hyperglycæmia caused by injection of a proportionate dose of adrenalin is well marked To find out how far the thyroid gland takes part in this response, the following line was adopted —

A series of 13 healthy albino Himalayan rabbits was selected, usual care being taken for housing and feeding them, and an insulin test was done on each

of these animals The dose of insulin being 1 Physiological Rabbit Unit (P R U) per kilo The results of the insulin test are given in Table I

TABLE I

Insulin *response in the albino Himalayan rabbits*
Before thyroidectomy
(Dose 1 P R Unit per kilo)

Serial number	Identification	Weight in grammes	Initial blood-sugar Per cent	Blood-sugar 2 hours after insulin injection Per cent
1	A H 1	1,390	0·102	0·068
2	A H 2	1,550	0 102	0·068
3	A H 3	1,800	0·112	0·068
4	A H 5	1,100	0 086	0·060
5	A H 6	1,370	0·072	0·052
6	A H 7	1,160	0·100	0·052
7	A H 8	1,200	0·095	0·056
8	A H 9	1,450	0·085	0 054
9	A H 11	1,590	0·107	0·053
10	A H 12	1,370	0·123	0·062
11	A H 13	1,530	0 100	0·048
12	A H 14	1,440	0·121	0·054
13	A H 15	1,330	0·112	0·076
AVERAGE			0·101	0·059

REMARKS

Average reduction of blood-sugar after insulin	41 6 per cent
Average dose of insulin given	1 4 P R U
Reduction of blood-sugar per each P R Unit of insulin	29·7 per cent

It will be seen from the above table that the average initial blood-sugar of these rabbits was 0 101 per cent and that 2 hours after insulin, it became 0·059 per cent, i e, an average reduction of 41 6 per cent which thus means a reduction of 29·7 per cent per each Physiological Unit of insulin

Adrenalin response in a series of 10 albino Himalayan rabbits was next tested, the dose of adrenalin given was 0 15 mg per kilo The following table gives the results of adrenalin response in the albino Himalayan rabbits

TABLE II

Adrenalin *response in albino Himalayan rabbits*

Before Thyroidectomy

(Dose 0·15 mg per kilo)

Serial number	Identification	Weight in grammes	Initial blood-sugar Per cent	Blood-sugar 1 hour after adrenalin injection Per cent
1	A II 1a	1,500	0·112	0·210
2	A II 2a	1,160	0·125	0·256
3	A II 3a	1,500	0·083	0·213
4	A II 4a	1,180	0·088	0·221
5	A II 5a	1,180	0·116	0·211
6	A II 6a	1,360	0·096	0·260
7	A II 7a	1,100	0·112	0·240
8	A II 8a	1,510	0·128	0·296
9	A II 9a	1,500	0·133	0·308
10	A H 10a	1,160	0·106	0·288
AVERAGE			0·110	0·256

REMARKS

Average rise of blood-sugar after adrenalin	132·7 per cent
Average dose of adrenalin given	0·214 mg
Rise of blood-sugar per each 0·1 mg of adrenalin	62·0 per cent

It will be seen from the above table that the average initial blood-sugar of these rabbits was 0·110 per cent and that 1 hour after adrenalin injection, it became 0·256 per cent, i.e., a rise of 132·7 per cent in 1 hour. *The rise of blood-sugar per each 0·1 mg of adrenalin was thus 62 per cent*

All these rabbits were next prepared for thyroidectomy, which was performed under ether anæsthesia, nearly the whole of the gland being removed, but care was taken to leave the parathyroids intact. The wounds healed up by first intention and the stitches were removed on the 8th day. About 7 more days were allowed to elapse to get the animals back to the normal state.

Insulin tests were again done on 8 of these thyroidectomized animals (of the A H group) using the same technique as before. The results are given in Table III.

TABLE III

Insulin response in albino Himalayan Rabbits

After thyroidectomy

(Dose 1 P R U per kilo)

Serial number	Identification as in Table I	Weight in grammes	Initial blood-sugar level Per cent	Blood-sugar 2 hours after insulin injection Per cent	REMARKS
1	A H 1	1 390	0 100	0 033	Severe hypoglycæmic convulsions, glucose given intravenously, saved
2	A H 2	1,550	0 104	0 036	Convulsions
3	A H 3	1,800	0 110	0·027	Severe convulsion, retraction of the head and extension of the hind limbs, intravenous glucose given but died in spite of it
4	A H 5	1,100	0 088	0·037	
5	A H 6	1,370	0·075	0 032	
6	A H 8	1,200	0·098	0 021	Very severe convulsions, died in spite of glucose
7	A H 9	1,450	0 088	0·035	Tremor, restlessness, rapid breathing
8	A H 15	1,330	0 114	0 042	Severe convulsions, relieved with intravenous glucose
AVERAGE			0 097	0·033	

REMARKS

Average reduction of blood-sugar after insulin	66 per cent
Average dose of insulin given	1 4 P R U
Reduction of blood-sugar per each P R Unit of insulin	47 1 per cent

The results in the above table are very striking (*cf* Table I) The same rabbits (Table I), which were very resistant to insulin, before thyroidectomy, had severe reactions after the operation with the same dose of insulin, and two of them died with typical hypoglycæmic convulsions

The average initial blood-sugar in these rabbits was found to be 0 097 per cent and 2 hours after insulin it became 0 033 per cent as against 0 059 per cent in the unoperated cases The average reduction of blood sugar was thus 66 per cent, or 47 1 *per cent per each physiological rabbit unit,* as against 29 7 per cent in the unoperated ones

In 6 of the thyroidectomized albino Himalayan rabbits (of the 'A H a' group) adrenalin response was tested, using the same technique and the same dose per kilo as before

TABLE IV

Adrenalin response in albino Himalayan rabbits

After thyroidectomy

(Dose 0 15 mg per kilo)

Serial number	Identification as in Table II	Weight in grammes	Initial blood-sugar Per cent	Blood-sugar 1 hour after adrenalin injection Per cent
1	A H 1a	1,500	0·106	0·180
2	A H 2a	1,160	0·101	0 186
3	A H 5a	1,180	0 088	0 146
4	A H 6a	1,360	0 074	0·135
5	A H 9a	1 500	0 083	0 128
6	A H 10a	1,160	0 120	0·188
AVERAGE			0·095	0·160

REMARKS

Average rise of blood-sugar after adrenalin	68·4 per cent
Average dose of adrenalin given	0·2 mg
Rise of blood-sugar per each 0·1 mg of adrenalin	34 2 per cent

The above results are also striking, as compared with those in Table II The same animals which, before thyroidectomy, gave such well-marked response in blood-sugar after adrenalin injection, gave a much poorer response after the operation

The average initial blood-sugar in these rabbits was 0 095 per cent and the same 1 hour after adrenalin injection became 0 160 per cent, i e, a rise of 68 4 per cent only, as against a rise of 132 7 per cent before thyroidectomy

The rise of blood-sugar in these rabbits per each 0 1 *mg of adrenalin* was thus 34 2 per cent as against 62 per cent in the same rabbits, before thyroidectomy

The following table (Table V) summarizes the main results of Tables I, II, III and IV and explains itself —

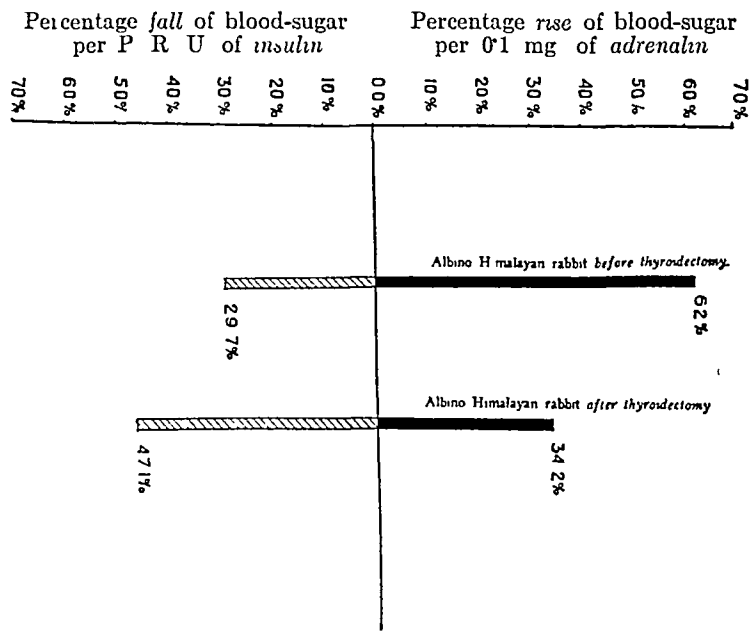
TABLE V

Showing the difference in insulin and adrenalin response in albino Himalayan rabbits before and after thyroidectomy

	ADRENALIN RESPONSE		INSULIN RESPONSE	
	Average rise of blood-sugar after adrenalin Per cent	Average rise of blood-sugar *per 0·1 mg of adrenalin* Per cent	Average fall of blood-sugar after insulin Per cent	Average fall of blood-sugar per physiological rabbit unit of insulin Per cent
I Albino Himalayan rabbits *before thyroidectomy* (Tables I and II)	132·7	62·0	41·6	29·7
II The same rabbits *after thyroidectomy* (Tables III and IV)	68·4	34·2	66·0	47·1

These results have been graphically shown in Graph 1

GRAPH 1



The dark lines above 0 represent the percentage rise of blood-sugar after adrenalin injection The interrupted lines below 0 represent the percentage fall of blood-sugar after insulin

The well-marked difference in results between the normal and thyroidectomized albino rabbits is thus quite evident, and clearly indicates that the thyroid takes a leading part in influencing the inter-relationship between insulin and adrenalin

We think the above experiments tend to show that the absence or deficiency of thyroid secretion *depresses* the function of the adrenals to an appreciable extent, but *enhances* the activity of insulin to a remarkable degree Thyroidectomy probably depresses the functions of the chromaphil tissues Normally, thyroid secretion is believed to be a direct stimulant to the chromaphil tissues, causing them to yield adrenalin to blood in larger quantity The excess of adrenalin thus secreted acts through the splanchnic nerve and the hepatic plexus and causes a release of sugar from the glycogen store-house of the liver

It is thus evident from the above experiments that thyroidectomy enhances the activity of the insulin-producing cells of the pancreas and alters the glycogenolytic response of the liver to adrenalin stimulation Some observers have noted that the inhibitory action of the thyroid on the pancreas is removed to a large extent after thyroidectomy and that there is a distinct increase in the islet tissues of the pancreas after thyroidectomy

Insulin and adrenalin responses in the brown Belgian-hare type of rabbits

Tables VI and VII give details of the insulin and adrenalin responses obtained in the pigmented variety of rabbits To summarize the results and

Table VI

Showing insulin response in Belgian-hare type of rabbits

(Dose 1 P R Unit per kilo)

Serial number	Identification	Weight in grammes	Initial blood-sugar Per cent	Blood-sugar 2 hours after insulin injection Per cent
1	B1 12	1,720	0·122	0 035
2	B1 13	1,877	0·133	0·052
3	B1 14	2,477	0 133	0 040
4	B1 15	2,557	0 120	0 040
5	B1 16	1,682	0 100	0·035
6	B1 17	1,985	0·100	0 040
7	B1 18	1 695	0 110	0 040
8	B1 19	1,727	0 100	0 045
9	B1 20	1,450	0 120	0·040
10	B1 21	1,342	0 135	0 045
Average			0 117	-0 011

REMARKS

Average reduction of blood-sugar	65 per cent
Average dose of insulin given	1·76 P R U
Reduction per unit of insulin	36·9 per cent

to compare them with those obtained in case of the albino type, Table VIII has been given

The results clearly indicate that these rabbits are very much more sensitive to insulin than the albino Himalayan variety All the rabbits had hypoglycæmic reactions after insulin, varying from a moderate to severe degree, and 3 out of 10 rabbits died in spite of intravenous injection of glucose

The average reduction of blood-sugar after insulin was 65 per cent, as against 41 6 per cent in the albino variety *The reduction of blood-sugar per each physiological rabbit unit of insulin* was thus 36 9 per cent as against 29 7 per cent of the albino variety (*cf* Table I)

TABLE VII

Showing adrenalin *response in Belgian-hare type of rabbits*

(Dose 0 15 mg per kilo)

Serial number	Identification	Weight in grammes	Initial blood-sugar Per cent	*Blood-sugar 1 hour* after adrenalin injection Per cent
1	Br 12	1,720	0·118	0 150
2	Br 13	1,877	0 128	0·175
3	Br 16	1,782	0 106	0 138
4	Br 17	1,985	0 108	0·140
5	Br 18	1,795	0 112	0 164
6	Br 19	1,727	0·103	0 155
AVERAGE			0 112	0 153

REMARKS

Average rise of blood-sugar	36 6 per cent
Average dose of adrenalin given	0·265 mg
Rise per 0·1 mg of adrenalin	13·8 per cent

The above table clearly indicates the poor response of these rabbits to adrenalin stimulation The rise of blood-sugar, after adrenalin injection, was only 36 6 per cent as against 132 7 per cent in the albino Himalayan variety *The percentage rise of blood-sugar per each* 0 1 *mg of adrenalin* in this variety was thus 13 8 per cent as against 62 per cent in the albino variety

TABLE VIII

Showing at a glance the differences in result of insulin and adrenalin responses in the albino Himalayan type and the brown Belgian-hare type of rabbits

(Dose of adrenalin given 0·1 mg per kilo of body-weight)

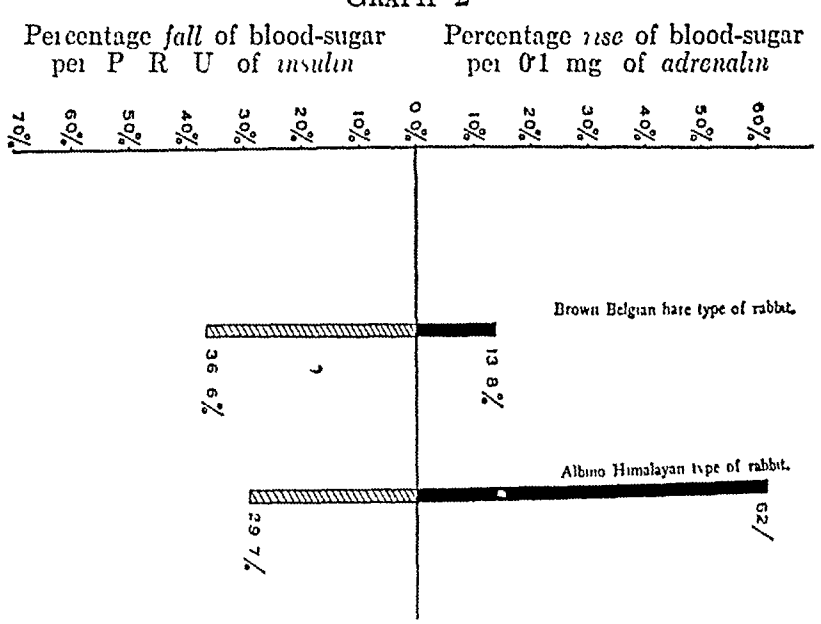
(Dose of insulin given 1 P R Unit per kilo of body-weight)

	ADRENALIN RESPONSE		INSULIN RESPONSE	
	Average rise of blood-sugar after adrenalin injection Per cent	Rise of blood-sugar *per* 0·1 *mg of adrenalin* Per cent	Average reduction of blood-sugar after insulin injection Per cent	Average reduction of blood-sugar per Physiological Rabbit Unit of insulin Per cent
Albino Himalayan type of rabbits (Tables IV and V)	132·7	62·0	41·6	29·7
Brown Belgian-hare type of rabbits (Tables VI and VII)	36·6	13·8	65·0	36·9

The well-marked differences in the results of adrenalin and insulin response in the two types of rabbits is very clearly represented in this table

These results have been graphically represented in Graph 2

GRAPH 2



The dark lines above 0 represent the percentage rise of blood-sugar after adrenalin injection The interrupted line below 0 represents the percentage fall of blood-sugar after insulin

It is a point worthy of note that the insulin response in the brown Belgian type of rabbits seems to resemble closely that of the albino Himalayan type of rabbits after thyroidectomy (*vide* Table III)

Summarizing the results of the above animal experiments, we come to the following broad conclusions —

(1) That the albino animals react *powerfully* to adrenalin
(2) „ „ „ „ „ *poorly* to insulin
(3) That the pigmented animals react *poorly* to adrenalin
(4) „ „ „ „ „ *powerfully* to insulin

Susceptibility in human subjects

To find out if similar results would ensue in human subjects, the writer proceeded to study the adrenalin and insulin response in human subjects In the first instance, a few healthy normal Indian subjects were selected and both the adrenalin and the insulin response in these individuals was studied The dose of insulin given was 1 clinical unit per each 10 kilo of body-weight, whereas the average dose of adrenalin was 0 5 mg The results of both the tests have been summarized in Table IX

Table IX

Showing the adrenalin *and* insulin *response in normal Indian subjects*

Case number as in experimental book	Initial blood-sugar level (mg per 100 c c)	Adrenalin response		Insulin response
		Maximum rise of blood-sugar after adrenalin injection Per cent	Maximum rise of systolic pressure after adrenalin injection Per cent	Maximum fall of blood-sugar after insulin injection Per cent
N 1 / 200	76	22·2	10·0	30·3
N 2 / 204	85	21 2	10·0	29 5
N 3 / 206	80	42·0	14 0	22 5
N 4 / 208	82	44 4	13 6	20 8
N 5 / 209	90	30 0	6 1	20·0
N 6 / 210	80	25 5	14 0	25 0
N 7 / 211	75	20·0	10·0	20·0
Average	81 1	29·3	11 1	24·0

From the analysis of the above table, we find that in normal healthy Indians —

(1) The average initial blood-sugar level is 81 1 mg per 100 c c
(2) The average rise of blood-sugar after adrenalin is 29 3 per cent
(3) The average rise of systolic pressure is 11 1 per cent
(4) The average fall of blood-sugar after insulin is 24 per cent

We next turned our attention to human subjects suffering from diseases where there is an alteration in the pigmentation of the skin Two groups of cases were selected —

(1) Diseases in which there is loss of pigmentation in varying degrees, e g, *leucoderma, albinism,* etc
(2) Diseases in which there is increased pigmentation We included in this series cases of *chloasma* and *kala-azar*

According to Dr Napier's observation on the pigmentation of skin in 250 cases of kala-azar, there was a slight increase of pigmentation in about 40 per cent of the cases and markedly so in about 5 per cent of the cases

The writer next proceeded to study the effects of adrenalin and insulin response in these groups of cases separately

Table X gives the summary of results of both these tests in cases of leucoderma

TABLE X.

Showing adrenalin and insulin response in cases of leucoderma

Serial number	Specification	Initial blood-sugar level (mg in 100 c c)	ADRENALIN RESPONSE		INSULIN RESPONSE
			Maximum rise of blood-sugar after adrenalin injection Per cent	Maximum rise of systolic pressure after adrenalin injection Per cent	Maximum fall of blood-sugar after insulin injection Per cent
1	L 1	57	113·5	12·8	12·2
2	L 2	64	68·7	16·0	16 2
3	L 3	60	63·3	25 0	5 0
4	L 4	55	80 3	11 6	12·8
5	L 5	55	53·3	18·6	18·1
6	L 6	80	51 1	23·3	18·7
7	L 7	79	60·7	10·2	17·2

TABLE X—*concld*

Serial number	Specification	Initial blood-sugar level (mg in 100 c c)	Adrenalin response		Insulin response
			Maximum rise of blood-sugar after adrenalin injection Per cent	Maximum rise of systolic pressure after adrenalin injection Per cent	Maximum fall of blood-sugar after insulin injection Per cent
8	L 8	65	61 5	12·5	18·1
9	L 9	60	61 6	11 6	14·2
10	L 10	73	58·9	21 0	19·6
11	L 11	81	56 7	11 1	20·0
12	L 12	105	36 0	16 6	4 7
13	L 13	72	47 2	11 1	16·0
14	L 14	75	22·6	17·0	18 0
15	L 15	60	50·0	16 4	13 9
16	L 16	55	54 0	Not done	15 8
17	L 17	100	26·0	14 8	15 2
18	L 18	61	50 8	13·2	19·2
19	L 19	95	57 8	12·7	19·5
AVERAGE		71 1	56 5	15 3	15 4

From analysis of the above table we find that in cases of leucoderma,

(1) The average initial (fasting) blood-sugar level is 71 1 mg per 100 c c

(2) The average rise of blood-sugar after adrenalin was 56 5 per cent

(3) The average rise of systolic pressure is 15 3 per cent

(4) The average fall of blood-sugar after insulin is 15 4 per cent

Table XI is a summary of adrenalin and insulin response in cases of kala-azar All the cases selected were verified by laboratory blood examinations and spleen punctures

TABLE XI

Showing adrenalin and insulin response in cases of kala-azar

Serial number	Specification	Initial blood-sugar (mg per 100 c c)	ADRENALIN RESPONSE		INSULIN RESPONSE
			Maximum rise of blood-sugar after adrenalin injection Per cent	Maximum rise of systolic pressure after adrenalin injection Per cent	Maximum fall of blood-sugar after insulin injection Per cent
1	K 1	78	11·0	6	29
2	K 2	93	20·0	10	52
3	K 3	70	15·0	No rise	55
4	K 4	100	17·0	16	29
5	K 5	100	15·0	No rise	26
6	K 6	110	11·0	2	34
7	K 8	97	18·0	7	30
8	K 9	102	12·0	16	16
9	K 10	90	10·0	5	6
10	K 11	95	17·0	18	33
11	K 12	80	20·0	25	28
12	K 13	75	13·0	3	42
13	K 14	80	15·0	No rise	19
14	K 15	90	22·0	5	55
15	K 16	100	13·0	No rise	20
16	K 17	81	9·0	8	40
17	K 18	85	6·0	No rise	42
18	K 19	80	10·0	8	39
19	K 20	99	19·0	8	66
AVERAGE		89·7	14·5	7·2	34·8

The analysis of the above table shows that in cases of kala-azar,

(1) The average initial (fasting) blood-sugar level is 89·7 mg per 100 c c
(2) The average rise of blood-sugar after adrenalin is 14·5 per cent
(3) The average rise of systolic pressure after adrenalin is 7·2 per cent
(4) The average fall of blood-sugar after insulin is 34·8 per cent

Table XII summarizes the results of adrenalin and insulin response in cases of chloasma in varying degrees of pigmentation

TABLE XII

Showing adrenalin and insulin response in cases of chloasma

Serial number	Specification	Initial blood-sugar level (mg per 100 c c)	ADRENALIN RESPONSE		INSULIN RESPONSE
			Maximum rise of blood-sugar after adrenalin injection Per cent	Maximum rise of systolic pressure after adrenalin injection Per cent	Maximum fall of blood-sugar after insulin injection Per cent
1	Chl 1	100	9·5	—1 6	39·0
2	Chl 2	90	14 5	5 0	44 5
3	Chl 3	95	13·5	1 2	34 8
4	Chl 4	64	17 4		26·5
5	Chl 5	80	23·7	3·5	47 1
6	Chl 6	90	3·7	—5 0	33 4
AVERAGE		86·5	13·7	0·6	37·5

An analysis of the above table shows that in cases of chloasma,

(1) The average initial blood-sugar level is 86 5 mg per 100 c c

(2) The average rise of blood-sugar after adrenalin is 13 7 per cent

(3) The average rise of systolic pressure is 0 6 per cent only

(4) The average fall of blood-sugar after insulin is 37 5 per cent

The results of Tables IX, X, XI and XII have been summarized in the following table which definitely show striking differences in results in these groups of cases as compared to normal —

TABLE XIII

(*Summarizes the results in Tables IX, X, XI, and XII*)

Cases	Initial blood-sugar level (mg per 100 c c)	ADRENALIN RESPONSE		INSULIN RESPONSE
		Average rise of blood-sugar after adrenalin Per cent	Average rise of systolic pressure after adrenalin Per cent	Average fall of blood-sugar after insulin Per cent
Normal	81 1	29·3	11 1	24·0
Leucoderma	71 1	56 5	15·3	14 9
Kala-azar	89·7	14 5	7 2	34 8
Chloasma	86 5	13·7	0·6	37 5

The above table clearly brings out some well-defined and striking facts It shows —

(1) That the cases of *leucoderma* show a well-marked adrenalin response as compared with normal The percentage rise of both blood-sugar and

TABLE XV

Showing the synopsis of results of adrenalin and insulin response in cases of kala-azar before and after treatment by antimony compounds The figures for normal are also given to compare results

Cases	Initial blood-sugar level (mg per 100 c c) Per cent	ADRENALIN RESPONSE		INSULIN RESPONSE
		Average rise of blood-sugar after adrenalin injection Per cent	Average rise of systolic pressure after adrenalin injection Per cent	Average fall of blood-sugar after insulin injection Per cent
Cases of kala-azar before treatment	89·7	14·5	7·2	34·8
The same cases after treatment	83·0	29·5	12·5	19·5
Normal controls	81·1	29·3	11·1	24·0

From an analysis of Table XV, we come to the following rather important findings —

(1) That the average initial (fasting) blood-sugar level in the non-treated cases of kala-azar is 89·7 mg per 100 c c whereas it is 83 mg in the same cases after treatment

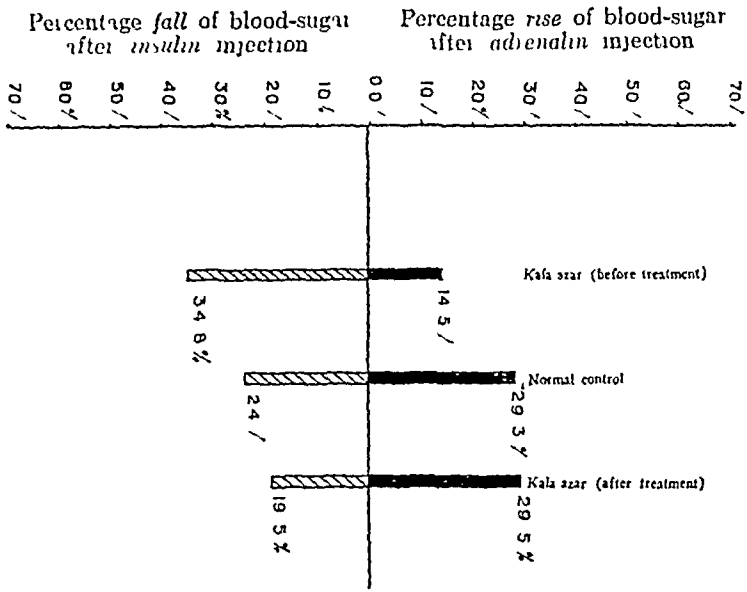
(2) That the maximum rise of blood-sugar after adrenalin injection in the non-treated cases is 14·5 per cent, which increases to 29·5 per cent after treatment, thus approaching normal This may be taken as indicative of an improvement in the adrenal function

(3) That the maximum rise of systolic pressure after adrenalin injection in the non-treated cases of kala-azar is 7·2 per cent only, which increases to 12·9 per cent after treatment This figure also approaches normal

(4) That the maximum fall of blood-sugar after insulin injection is 34·8 per cent in the non-treated cases, which decreases to 19·5 per cent after treatment, and approaches near normal The lowering of the high insulin response after treatment is, it is believed, due to the improved adrenal function, which causes more release of sugar from the glycogen store-house of the liver, and thus neutralizes the action of insulin to a large extent.

To bring out the differences between adrenalin and insulin response in cases of kala-azar before and after treatment by antimony compounds graphically and to compare them with a normal control, Graph 4 is appended below

GRAPH 4



SUMMARY AND MAIN CONCLUSIONS

(1) The action of adrenalin and insulin on the blood-sugar of rabbits, when these happened to be of the white, brown and coloured variety, was investigated It was found that, whereas, the blood-sugar rise per 0 1 mg of adrenalin was 62 per cent in the albino rabbits, it was only 13 8 per cent in the brown variety Also, whereas, insulin caused a fall of 29 7 per cent of blood-sugar per P R Unit in the albino, it caused a greater fall (i e, 36 6 per cent) in the brown variety

(2) That the thyroid takes a leading part in influencing the inter-relationship between the action of adrenalin and insulin was shown by the fact that the adrenalin response causing a 62 per cent blood-sugar rise in the albino rabbits dropped to 34 2 per cent after thyroidectomy, also that the insulin response causing a blood-sugar fall of 29 7 per cent increased to 47 1 per cent after the operation

(3) The adrenalin and insulin response in human subjects suffering from diseases influencing the pigmentation of the skin was studied These fall into the following groups —

(*a*) Leucoderma, albinism—loss of pigmentation

(*b*) Chloasma, kala-azar—increased pigmentation

A group of normal healthy Indians were treated as controls

It was found (*i*) that adrenalin caused a much higher rise in blood-sugar and also of systolic pressure in the leucoderma cases as compared with that of the other group, as well as the normal

(*ii*) That insulin caused a greater reduction of blood-sugar in the pigmented cases as compared with the leucoderma and the normal cases

(*iii*) That cases of leucoderma and albinism behaved more or less like the albino type of rabbits as regards the adrenalin and insulin responses

(*iv*) That cases showing increased pigmentation behaved more or less like the pigmented rabbits

(4) A group of cases of kala-azar of varying degrees of severity which gave a very poor adrenalin response before treatment were next selected, and adrenalin and insulin response were tested again after a course of injection of antimony compound to see if these responses would be influenced thereby It was found that, whereas, adrenalin caused a rise of 14 5 per cent of blood-sugar and 7 2 per cent of systolic pressure before treatment, it rose to 29 5 per cent and 12 5 per cent respectively after treatment, definitely indicating an improvement in the function of the supra-renal glands Also, whereas before treatment, insulin caused a greater fall (34 8 per cent) in the blood-sugar, it was much reduced (19 5 per cent only) after treatment, also showing that the improved adrenal function caused a greater release of sugar from the glycogen store-house of the liver, which neutralized the action of insulin on blood-sugar to a large extent

The influence on the texture of the skin will be treated in a separate paper

GLUCOSE- AND LÆVULOSE-TOLERANCE CURVES IN VARIOUS CONDITIONS

BY

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THE ingestion of 50 grammes of glucose by a normal individual leads to a rise in blood-sugar which subsides to the initial level or below it in one and a half to two hours. The highest value attained is less than that at which sugar usually appears in the urine (0·18 to 0·20 per cent.). Although the liver plays the chief part in thus regulating the blood-sugar level, defective tolerance to glucose results from pathological changes in other tissues which take part in carbohydrate metabolism, notably the pancreas and muscles, and a renal glycosuria may interfere with the shape of the glucose-tolerance curve. Apart from diabetes mellitus and allied disorders, intolerance to glucose has been found in other diseases of the endocrine glands, in non-endocrine obesity (Goldblatt, Forrest Smith and Gardiner Hill, 1928), in certain skin diseases (Somerford, 1929), in upper and lower neuron paralyses and myasthenia gravis (Payne and Hale-White, 1927), in acute nephritis and nephritis with urea retention (Major, 1923, William and Humphreys, 1919), in cirrhosis of the liver (Strouse, 1920), in rheumatoid arthritis (Pemberton and Tompkins, 1920) and in other conditions. Bhatia and Coelho (1925) found abnormally high blood-sugar levels in normal Indians in Bombay after administration of 50 grammes of glucose or cane sugar. In some individuals the fasting blood-sugar was also above the normal figure. Non-vegetarians had a lower average blood-sugar and greater carbohydrate tolerance than vegetarians. Payne and Hale-White (1927) investigated the diagnostic value of the glucose-tolerance curve and showed that the only disease in which a diagnosis could be made

from the curve alone was diabetes In other cases in which the clinical evidence leads to doubt the glucose-tolerance test may be of value in differentiating a condition in which the curve is usually normal from one in which it is generally abnormal

As regards the metabolism of lævulose it is generally agreed that the liver is the only organ concerned in the storage of this sugar, and hence inability to dispose of lævulose is taken to indicate defective glycogenic function of the liver In a normal adult there is little or no change in the blood-sugar following the oral administration of 50 grammes of lævulose Talleiman (1923) gives the following standards of normality for this test —

(1) The blood-sugar should not exceed 0 135 per cent nor show a rise of 30 milligrams above the initial fasting level

(2) The raised blood-sugar should not persist beyond 1½ or at most 2 hours

This observer found the renal threshold for lævulose to he between 0 115 per cent and 0 13 per cent

The experiments recorded in this paper were carried out mostly in the cold weather on a number of patients in the medical wards of the Mayo Hospital, Lahore Defective storage of glucose had been noticed from time to time in the course of routine observations on such patients and the object of the present investigation was to obtain information regarding the frequency of this condition and the diseases with which it was associated Glucose-tolerance tests were carried out on 49 cases and lævulose tests on 29 Circumstances did not permit of both tests being done on the same individuals except in two instances The results are given in tabular form (Tables I and II) No cases of diabetes or other endocrine diseases are included The pneumonia patients examined were convalescent and hence may be regarded as practically 'normal' Blood for examination was obtained by pricking the finger and blood-sugar was estimated by McLean's method

The results show that nearly all the patients tested with glucose gave curves which were abnormal in one or more respects No type of curve, however, was diagnostic of any particular disease This confirms the finding of Payne and Hale-White Of the total cases examined (78), 52 had initial blood-sugar values above 0 120 per cent, a figure which is generally considered to be the upper limit of normality Included in these are 8 of the 10 convalescent pneumonia patients who may be considered to have been free from active disease at the time of examination We have not had an opportunity of estimating the blood-sugar in a series of normal Indians in the post-absorptive state but from isolated observations we consider a high value not to be uncommon The patients examined lived on a diet mostly vegetarian, especially the Hindus, but rigid vegetarianism is not common among these people

If the results of the lævulose-tolerance tests in Table II are judged by Tallerman's standards they indicate defective glycogenic function of the liver in all except one case Even if the maximum rise in the blood-sugar is alone

TABLE I

Glucose-tolerance curves

No	Sex	Age	Religion	Blood-sugar after giving 50 grammes dextrose					Clinical notes
				Initial	½ hour	1 hour	1½ hours	2 hours	
1	M	40	M	0·162		0·231	0·221	0·221	Chronic amœbic dysentery
2	„	45	„	0·164	0·211	0·190	0·209	0·181	Acute bacillary dysentery
3	„	50	„	0·085	0·132	0·134	0·169	0·173	Chronic amœbic dysentery
4	„	45	„	0·141	0·213	0·174	0·196	0·193	Diarrhœa, ?Bacillary dysentery
5	„	40	„	0·145	0·181	0·218	0·221	0·221	Convalescent broncho-pneumonia
6	„	25	H	0·151	0·207	0·231	0·195	0·150	Do
7	„	20	„	0·115	0·172	0·147	0·141	0·143	Do
8	„	26	M	0·132	0·170	0·159	0·155	0·159	Do
9	„	50	H	0·133	0·166	0·141	0·154	0·140	Do
10	„	28	„	0·135	0·219	0·264	0·227	0·182	Convalescent lobar pneumonia
11	„	28	M	0·104	0·137	0·164	0·193	0·184	Do
12	„	25	„	0·139	0·218	0·229	0·263	0·191	Atrophic cirrhosis of the liver
13	„	40	„	0·109	0·122	0·167	0·221	0·196	Do
14	„	20	H	0·127	0·189	0·191	0·181	0·204	Ankylostomiasis, spleen palpable, slight jaundice
15	„	18	M	0·164	0·156	0·209	0·213	0·177	Ankylostomiasis
16	„	20	„	0·128	0·170	0·185	0·160	0·154	Do

TABLE I—*concld*

No	Sex	Age	Religion	Blood-sugar after giving 50 grammes dextrose					Clinical notes
				Initial	½ hour	1 hour	1½ hours	2 hours	
17	M	25	M	0·100	0·137	0·149	0·139	0·122	Ankylostomiasis
18	„	24	H	0·110	0·133	0·135	0·116	0·119	Do
19	„	30	„	0·141	0·163	0·191	0·213	0·218	Sciatica Pyorrhœa alveolaris
20	„	30	„	0·117	0·141	0·181	0·169	0·173	Sciatica
21	„	22	M	0·153	0·191	0·184	0·229	0·096	Do
22	„	16	H	0·137	0·193	0·221	0·187	0·166	Polyarthritis
23	„	25	M	0·110	0·150	0·156	0·156	0·159	Do
24	„	20	H	0·117	0·231	0·212	0·175	0·120	Do (Gonorrhœal)
25	„	40	M	0·150	0·171	0·181	0·167	0·118	Do ?Rheumatic
26	„	33	„	0·123	0·190	0·196	0·148	0·134	Neuralgia, chronic gonorrhœa
27	„	30	H	0·113	0·187	0·181	0·184	0·181	Facial paralysis
28	„	20	„	0·125	0·162	0·141	0·179	0·137	Do
29	„	20	„	0·085	0·136	0·136	0·156	0·127	Tubercular pleurisy
30	„	35	M	0·159	0·209	0·204	0·201	0·170	Do
31	„	30	„	0·114	0·113	0·187	0·177	0·222	Tabes mesenterica
32	„	38	H	0·136	0·172	0·181	0·187	0·162	Do
33	„	25	„	0·106	0·168	0·131	0·115	0·115	Tubercular enteritis

34		40	„	0·164	0·201	0·243	0·210	0·166	Pulmonary tuberculosis Four years' duration
35		25	H	0·121	0·216	0·216	0·200	0·184	Chronic pulmonary tuberculosis
36	„	25	M	0·162	0·189	0·166	0·179	0·181	Chronic malaria
37	„	40	„	0·104	0·152	0·191	0·193	0·179	Do Spleen palpable
38	„	20	„	0·098	0·150	0·172	0·143	0·111	Do
39	„	25	„	0·118	0·122	0·136	0·152	0·109	Do
40	„	21	„	0·102	0·181	0·156	0·092	0·139	Enteric fever
41	„	30	„	0·122	0·266	0·256	0·271	0·121	Renal colic
42	„	35	„	0·102	0·115	0·233	0·174	0·154	Chronic nephritis
43	„	25	M	0·175	0·191	0·164	0·162	0·156	Carbon monoxide poisoning
44	„	25	H	0·094	0·146	0·160	0·169	0·148	Myeloid leukæmia
45	„	28	M	0·112	0·116	0·211	0·172	0·170	Epilepsy
46	„	15	„	0·162	0·193	0·211	0·221	0·193	Idiopathic œdema (Milroy's disease)
47	„	35	H	0·143	0·181	0·198	0·224	0·193	Chronic myocarditis
48	„	16	„	0·146	0·216	0·236	0·168	0·201	Trauma of chest
49	„	50	„	0·172	0·253	0·266	0·229	0·286	Cardiac irregularity (frequent premature contractions)

TABLE II

Lævulose-tolerance curves

Number	Sex	Age	Religion	Blood-sugar after giving 40 grammes of lævulose					Silvanoff's test in urine		Maximum rise of blood-sugar mg per cent	Bilirubin units in plasma after 2 hours	Clinical notes
				Initial	½ hour	1 hour	1½ hours	2 hours	1 hour	2 hours			
1	M	20	M	0·123	0·135	0·134	0·116	0·116	Neg	Neg	12	0·85	Boils on face and arms
2	„	40	H	0·120	0·135	0·130	0·131	0·132	Pos	Pos	15	0·12	Chronic pulmonary tuberculosis
3	„	50	M	0·139	0·154	0·143	0·137	0·139	Neg	Neg	15	Trace	Hemiplegia
4	„	34	„	0·149	0·160	0·164	0·151	0·147	Pos	Pos	15	„	Psoriasis
5	„	30		0·143	0·147	0·159	0·152	0·150			16	„	Convalescent broncho-pneumonia
6	„	35	H	0·199	0·201	0·216	0·166	0·164	Pos	Pos	17	„	Chronic pulmonary tuberculosis
7	„	35		0·122	0·133	0·139	0·126	0·141	Neg	Neg	19	0·7	Meningo-myelitis (syphilitic)
8	„	33	M	0·134	0·153	0·150	0·136	0·140	,,	,,	19	0·65	Chronic pulmonary tuberculosis
9	„	36	„	0·139	0·143	0·160	0·149	0·146	Pos	Pos	21	0·43	Do do do
10	„	30	„	0·115	0·135	0·136	0·125	0·120		„	21	0·57	Tubercular pleurisy
11	„	36	H	0·104	0·128	0·117	0·106	0·097	Neg		24	0·82	Ankylostomiasis
12	„	18		0·115	0·140	0·135	0·135	0·123	„	Pos	25	0·55	Influenza—convalescent
13	„	28	M	0·156	0·181	0·168	0·147	0·149		„	25	Trace	Chronic pulmonary tuberculosis
14	„	30		0·127	0·152	0·146	0·137	0·131	Neg	Neg	25	0·53	Convalescent lobar pneumonia
15	„	25	H	0·121	0·146	0·142	0·140	0·133	Pos	Pos	25	Nil	Chronic parenchymatous nephritis

16	„	36	M	0·140	0·158	0·148		0·168	„	„	28	1·2	Catarrh of frontal sinuses
17	„	27	„	0·141	0·158	0·173	0·143	0·138	„	„	32	Trace	Tubercular enteritis
18	„	35	H	0·103	0·131	0·136	0·122	0·110			33	0·5	Progressive muscular atrophy
19	„	55	M	0·128	0·152	0·147	0·148	0·162			34	Trace	Chronic malaria, spleen palpable
20	„	55	„	0·139	0·173	0·159	0·162	0·150			31	„	Ringworm
21	„	30	H	0·132	0·168	0·156	0·150	0·145	Pos	Pos	36	„	Chronic malaria
22	„	40	M	0·131	0·156	0·177	0·158	0·146			36	Nil	Tubercular enteritis
23	„	35	„	0·127	0·151	0·149	0·164	0·116	Pos	Pos	37	0·67	Sciatica
24	„	36	H	0·134	0·171	0·164	0·152	0·139	„	„	37	1·8	Intestinal colic
25	„	16	„	0·133	0·174	0·165	0·145	0·131	Neg	Neg	41	Trace	Chronic polyarthritis
26	„	30	„	0·118	0·160	0·161	0·149	0·141	Pos	Pos	46	„	Sciatica Pyorrhœa alveolaris
27	„	25	M	0·134	0·152	0·152	0·181	0·139	Neg	Neg	47	1·7	Chronic malaria
28	„	20	„	0·133	0·163	0·174	0·189	0·147	„	Pos	56	Nil	Ankylostomiasis
29	„	40	„	0·137	0·175	0·199	0·187	0·149	„	Neg	62	Trace	Convalescent lobar pneumonia

considered this function must be adjudged abnormal in 13 As the patients in both series were suffering mostly from the same types of disease and were in other respects similar, it may be concluded that inefficiency of the liver in regard to glycogen formation and storage was in most of the cases to a greater or less extent responsible for the intolerance to carbohydrates This conclusion is supported by the fact that in the two subjects (Nos 19 and 22—Table I and Nos 26 and 25—Table II) on whom both tests were carried out there was intolerance to both sugars In certain diseases, e g , hepatic cirrhosis, the liver may have been the chief, or the only, organ at fault, but in others this was probably not so Defective circulation in the muscles (Pemberton, Cajori and Crouter, 1926), abnormal nervous influences or a predominantly vegetarian diet probably played a part in some instances Further, in view of the results obtained on the patients convalescent from pneumonia, the diseases from which our subjects were suffering at the time of examination may not have been the sole cause of their defective carbohydrate metabolism

One factor common to the patients examined is that they had all suffered from malaria from infancy, in greater or less degree, and in most cases had little or no effective treatment The bilirubin content of the plasma was estimated in the second series and with the exception of three patients (Nos 16, 24 and 27) all gave figures within, or close to the normal range (0 2 to 0 5 unit) This shows that there was no interference with the ability of the liver to excrete bile pigment and no considerable degree of blood destruction in the majority at the time of examination It is possible, however, that previous malaria may have produced a diminution in the glycogenic function of the liver which subsequent disease brought into evidence Sinton and Hughes (1924) showed that ordinary acute attacks of this disease may give rise to diminished lævulose-tolerance and Williams (1927) found markedly abnormal lævulose curves during the course of therapeutic malaria in patients suffering from G P I Another disease which may have caused hepatic injury in some of our cases is amœbiasis, a condition which is common among such people

Conclusion

Glucose- and lævulose-tolerance tests were performed on 49 and 29 hospital patients, respectively, suffering from various diseases No cases of diabetes or other endocrine disturbances were examined According to the usual standards the curves were abnormal in almost all cases The initial blood-sugar was high (above 0 120 per cent) in 52 The cause of these findings is discussed

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JUNGLE IN RELATION TO MALARIA IN BENGAL

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THE term 'Jungle-fever' as commonly used refers to a severe attack of malaria and it is a common belief among the lay public (and not an infrequent one among medical men as well) that jungles are malariogenic We frequently hear of jungle being intensely malarious, and that a single excursion into the jungle would be enough to bring on an attack of malaria In certain localities, the local people attribute the incidence of malaria to the proximity of the jungle and they believe that a clearance of the jungle would reduce malaria Some medical men have strong views on this point and would trace an attack of malaria to a visit to the jungle They have pressed for a clearance of jungle for a mile all around the village or habitation as a protection against malaria

My observations on the jungles in the province of Bengal show that they are not connected with malaria at all, but on the other hand actually form a protection against the breeding malaria transmitting species of *Anopheles* It will be of interest therefore to record these observations on the different jungle areas in Bengal

Some misapprehension may occur in regard to the term 'jungle' In using this term in the present article, I mean an uninhabited land covered by forest trees, shrubs or thick brushwood, tall and thick enough to produce shade and prevent the exposure of the land surface to sunlight to any appreciable degree The height of the trees and shrubs is not of importance as they may be very high or quite low The vegetation may have been artificially planted or it may be composed entirely of natural jungle flora Scrub-jungle consisting of straggling low-lying shrubs, pastural jungle, and savannahs do not come under the term 'jungle' as discussed in the present article The undergrowth of weeds and clumps of bamboo commonly occurring in the vicinity of houses and villages in Deltaic Bengal and locally known as *jangal* does not come under this category and is not discussed in the present paper

Jungle regions in Bengal

Jungles occur in different parts of Bengal We have the hill jungles of the mountains on the north, we have also the submontane jungles in the *Terai* and the *Duars*, the foot-hill zone Lastly, we have the mangrove jungles in the Sunderban area, the estuarine zone of Bengal The submontane jungle is that which comes in for much blame as being malariogenic since the submontane zone is intensely malarious The Sunderban jungle is less frequently blamed as being responsible for the malaria in the neighbourhood But no complaints are heard with regard to the jungle in the hill areas, there being very little malaria in the hill zone

Hill jungle

Montane jungles were investigated on three occasions The extent of breeding in these jungles is small and is restricted to the rainy season Four species of *Anopheles* occur in the hill jungle in Bengal, namely, *A gigas* var *simlensis, A aitkeni, A lindesayi* and *A annandalei,* and none of them has been known to transmit malaria Owing to the elevation, however, there is little or no malaria in the hill region and as such the question of the influence of the hill jungles on the local malaria does not arise

Submontane jungles

Submontane jungles occur throughout the foot-hill region in Bengal and cover parts of the *Terai* on the west and the *Duars* on the east This tract was at one time covered by dense natural jungles of the type of the 'tropical monsoon forests' They have been extensively cleared for purposes of cultivation of the land, chiefly for tea plantations The submontane zone has a rich anopheline fauna and it is notorious on account of its malariousness

The question of *Anopheles* breeding inside the jungles in the submontane zone has been investigated in some detail since it is not infrequently that one hears of the unhealthiness of an area as being due to the proximity of the jungle As the matter is concerned with the health of a large population consisting of labour and staff employed in the tea industry and in forestry, several areas in this zone have been investigated in detail to obtain accurate information about the anophelines breeding inside the jungles and those breeding in the cleared areas The results of these investigations are discussed below

1 A large belt of jungle land occurs on the north of Meenglas and Dalmkote, two adjacent tea plantations situated in the hyperendemic area in the Duars This jungle is quite close to many of the labour lines of the estates and during the rainy season the jungle has numerous streams It was desired to ascertain if the jungle was in any way responsible for the high incidence of malaria on these estates The anophelines breeding inside the jungle were investigated during different seasons for several years and the species found within the jungle were *A aitkeni, A barbirostris* and *A leucosphyrus,* the last mentioned species breeding in stagnant pools *A aitkeni* and *A barbirostris*

were the only species found in streams within the jungle, out in the open, the same streams were found to breed many other species, for example, *A maculatus*, *A minimus*, *A jeyporensis*, *A culicifacies*, *A jamesi* and *A majidi* The innocuous forest species breeding in the streams in the jungle are replaced, when the streams emerge into the open, by species known to be malaria transmitters or suspected to be such These observations are interesting as showing how a jungle is perfectly healthy in contrast to the open area which is decidedly malarious There is further support to this in the fact that the labour lines situated closest to the jungle are less unhealthy and have a lower spleen rate than those situated in the centre of the plantation The explanation of this lies in the fact that in the case of lines situated on the forest edge, the breeding of the carrier species occurs only on one side, while on the forest side there is none On the other hand, lines situated in the middle of the open area have breeding of the carrier species occurring on all sides

2 Madarihat in the Duars, situated in a clearing in the jungle area is an intensely malarious place Its population has been getting thinner on account of the unhealthiness of the station and local opinion pointed to a jungle area in the vicinity of Madarihat as being responsible for the malariousness of the station A survey of the station area and its vicinity showed that in the open area outside the jungle, *A minimus*, *A maculatus*, *A culicifacies*, *A fuliginosus*, *A sinensis*, *A vagus* and *A barbirostris* were quite common, while within the jungle, *A barbirostris* and *A leucosphyrus* only were found The anophelines breeding in the open include several species which are well-known carriers, while the species found inside the jungle are not concerned with the transmission of malaria, at least so far as India is concerned

This investigation shows the innocuousness of the jungle from the point of view of output of carrier anophelines and as such, jungle cannot be regarded as causing the malariousness of the station The age of the jungle does not seem to produce any difference in regard to the extent to which anophelines breed In this connection, jungles of varying ages, from five years onwards to 25 years old were investigated at Madarihat and at Sukna and they seem to behave entirely alike in preventing the breeding of carrier species

3 Some detailed work was carried out at Sukna, a small station in the Darjeeling Terai in the submontane zone, regarding the influence of jungle on species of *Anopheles* *Anopheles* surveys of the jungle area were carried out on several occasions during 1928 and 1929 and it was found during each of these surveys that the species of *Anopheles* found inside the jungle were different from those found in the cleared area Inside the jungles were found breeding, in streams *A aitkeni*, *A aitkeni* var *insulæflorum* and *A barbirostris*, in ditches and depressions *A leucosphyrus*, *A aitkeni*, *A aitkeni* var *insulæflorum* and *A barbirostris*, and in tree-holes *A annandalei* In the cleared area out in the open, these species were replaced by species like *A maculatus*, *A minimus*, *A maculipalpis*, *A fuliginosus* and *A philippinensis*, all of which are known transmitters of malaria If we follow up the course of a stream,

it is easy to demonstrate how in the open area malaria transmitting species are very common, while the same stream inside the jungle breeds an entirely different set of anophelines Whenever deforestation is carried out, the harmless jungle species of *Anopheles* disappear and a total change in the anopheline fauna of the water is brought about by the clearing of the jungle

4 Similar observations have been made at other places close to Sukna situated in the submontane zone, e g , Kadma, Bengdubi and Sevoke

5 At Rajabhatkhawa (Jalpaiguri district), a locality notorious on account of its malariousness and which has a spleen rate of above 90 per cent, it was interesting to find how at the edge of the jungle and the open area, heavy breeding of *A maculatus* and *A minimus* occurred, while a few yards within the jungle the breeding of these species stopped altogether

6 At Samsing Tea Estate (Duars) which is at an elevation of 1,800 feet, a dense jungle exists on the north and west of the estate The jungle area was entirely free from *A maculatus* and *A minimus* which were found breeding in the open area, the species of *Anopheles* found within the jungle being *A lindesayi* and *A aitkeni* which are not usually if ever associated with malaria On this estate, there are two coolie lines, one which is close to the forest boundary on the north and the other which is situated in the centre of the plantation The spleen rate of the lines near the forest is much lower than that of the other lines and the former is decidedly the healthier one

7 The District Medical Officer stationed at Rangamati, the headquarters station of the Chittagong Hill Tracts drew attention to the intense malariousness of the station and expressed his belief that a dense jungle close by was responsible for the high incidence of malaria at Rangamati He suggested that in the interests of public health, the jungle should be cut down and the area opened up in order to reduce the incidence of malaria In connection with this proposal, a malaria survey of the station and the neighbourhood was carried out by me and showed that the place was very malarious indeed I had found the spleen index of children within the station to be 70 per cent and it was as high as 90 per cent in a village adjoining it An anopheline survey of the station area and of the jungle showed that the jungle blamed on account of the malariousness of the locality was perfectly innocuous as regards breeding of carrier species On the other hand, within the station itself there were innumerable breeding places in the form of seepages, streams and ponds which were breeding anophelines in large numbers, some of the species found there being well-known carriers, the species found breeding within the station were *A karwari, A fuliginosus, A philippinensis* and *A jamesi* As a result of this investigation, it was recommended that instead of clearing the jungle as suggested by the local medical officer, it would be advantageous not only to maintain the jungle intact, but even to extend it so as to cover all the valleys and thereby render the seepages and streams unfit for the breeding of carrier anophelines

Mangrove jungles

The Sunderbun jungle area was investigated to determine the anophelines breeding within the jungle area and the influence of the jungle on the local malarial incidence The Sunderbans is a maze of islands cut up by a network of tidal channels Part of the area has been cleared of jungle and under rice cultivation, while part of it is still covered by dense jungle As most of the land is below spring tide level, the area is subject to tidal flooding except where it is artificially protected by means of embankments as in parts of the opened up area

The Sunderban jungle is a typical mangrove formation and differs from other jungles discussed previously both in the type of vegetation and its ecology The flora is characteristic in that it consists largely of species which are viviparous, have stilt roots and pneumatophores as adaptations against the flooding of the land and the absence of aeration of the soil The typical plants of this region are species of *Rhizophora, Ceriops, Kandelia, Bruguiera, Lumnitzera, Sonneratia, Carapa* and *Egiceras,* besides *Avicennia officinalis, Acanthus ilicifolius* and the stemless palm *Nipa fruticans*

It is only during exceptionally high spring tides that the forest land is entirely submerged, but ordinarily, it is only the lower regions of the forest that are covered by the flood Within the Sunderban jungle, there are some shallow basins which hold up brackish water or saline water and which are not affected by the tides These jungle swamps were investigated as well as the numerous small streams and channels within the forest In none of these were any anophelines found although a thorough search was made for them

The staff of the Forest Department posted in the interior of the Sunderban jungle stay in small floating flats anchored in creeks and channels Their rooms were searched for adult anophelines and it was surprising to find that no anophelines could be found even after a careful search A class of forest workmen known as the *Boah* live in the interior of the Sunderban forest in isolated huts erected on piles A search was made in a large number of these huts for anophelines and not even a single anopheline could be found in any of them

These results are in striking contrast to my experience in the cleared area The cleared land which is under cultivation is protected from the tides, which would destroy the crops, by means of embankments alongside every watercourse Within the cleared and protected area, heavy breeding of *A ludlowi* and *A subpictus* often in association with *A vagus* was observed in almost every place investigated Breeding in these places is very heavy and the number of adult anophelines very great The breeding places are the collections of brackish water which cannot drain into the watercourse which may be quite close, since such drainage is prevented by the embankments protecting the land against tidal flooding The breeding being heavy and the number of breeding places being numerous, it is easy to explain the high incidence of adult anophelines in the Sunderban cleared area One example may be given here

to illustrate the prevalence of adult anophelines Cattle are not able to stay out after sunset as they are so heavily pestered by the mosquitoes that they run into the cattle sheds for shelter early in the evening These cattle sheds are so constructed as to be capable of protection against mosquitoes They are of the closed type and are provided with a few small windows usually screened with mosquito gauze In addition to such protection, almost every cattle shed is smoked in the evenings to drive away the mosquitoes and the doors are then closed These precautions afford the cattle considerable protection against the mosquitoes I have not seen any other part of Bengal where so much precaution is taken for the protection of live stock against mosquitoes It should be superfluous to state that every person staying in this area, even the very poor, is provided with a mosquito curtain and the people take care to get under the curtain as early as possible after sunset The contrast between such a condition and the entire immunity from anophelines observed within the jungle area is indeed most striking

Whenever the writer, whilst engaged in the survey of the Sunderbans, halted for the night within the cleared area or close to it, the boat in which he was staying was invaded by hordes of anophelines, mostly *A ludlowi* and *A subpictus* But when he halted for the night in the interior of the jungle area he found no evidence of any anophelines whatsoever He took advantage of this experience and even when engaged in a survey of the cleared area, after finishing the day's survey work, he took the boat into the forest area towards the evening and halted for the night in the interior of the jungle and thus entirely avoided the anophelines

Although other species of *Anopheles* such as *A sinensis*, *A barbirostris*, *A fuliginosus* and *A varuna* are found in parts of the cleared area, *A ludlowi*, *A subpictus* and *A vagus* are perhaps the most common species of the cleared area There is no doubt that a considerable amount of malaria occurs in parts of the Sunderban cleared area and the malariousness is undoubtedly due to *A ludlowi*

In common with other jungle areas studied in Bengal, the mangrove jungle is not associated with any malaria prevalence The present investigations show that the Sunderban jungles are remarkably free from anophelines and therefore perfectly healthy Actually, the jungle forms a definite protection against the breeding of *A ludlowi*, the important carrier species of the region The cleared areas on the other hand abound with this species and some parts are very malarious

Discussion

In every type of jungle land investigated in Bengal, the anophelines found breeding within the jungle are entirely different from those found outside the jungle in the cleared area While the species found inside the jungle are such as are not usually found concerned in the transmission of malaria, those that occur in the cleared area are largely species definitely incriminated in regard

to the transmission of malaria The jungles as the present investigations show, are perfectly innocuous and actually form a definite protection against the breeding of harmful species of *Anopheles* Immediately the jungle is cut down and the land exposed to sunshine the malaria transmitting species of Anopheles commence to breed in places where previously there was no breeding at all or the breeding was only of such of the species as are quite harmless to man

The factors that account for the difference in the anopheline fauna are not yet clearly understood The important factor that inhibits the breeding of the carrier anophelines seems to be the dense shade that prevails inside the jungle and the absence of sunlight in its effect on the flora of the water But this is only a speculation as actually nothing is known definitely on this point But certain observations seem to point to shade not being the only factor concerned in the inhibition of the open breeders inside the jungle I have seen some areas in the interior of the Sukna forest where the water is exposed to the sun and conditions are apparently quite favourable for the open breeding species to establish themselves, but yet they are found there A small clearing in the interior of a jungle far removed from human habitation and the exposure of the water to sunshine without the modification of the jungle conditions caused by the presence of human dwellings in the clearance so made, do not seem to make any difference in the anopheline fauna of the water If the clearing be made and habitations are placed on the cleared land, the carrier species start breeding immediately The breeding of the open breeder species thus seems to be connected with two factors (*a*) the factor favourable for the larva, namely, the exposure of the water to sunshine and the consequent changes brought about in the fauna and flora of the water, and (*b*) the factor favourable for the adult mosquito in the availability of man and his domestic animals to feed on A combination of these two factors seems to be necessary for the breeding of the open breeders Either of these two factors by itself does not seem to be enough

Considered from the biological point of view, these observations on the innocuousness, as regards malaria, of jungles seem to be perfectly natural The transmitters of an entirely human parasite such as the malaria parasites of man are, would ordinarily be species which are closely associated with man A species living away from man with very little chance of feeding on man to any appreciable degree would not be expected to be responsible for the transmission of the parasite The species of *Anopheles* known to transmit malaria parasites in nature are all species closely associated with man or with his activities Jungle anophelines which breed inside the jungle and normally feed on jungle animals have ordinarily very poor chances of obtaining human blood and much less chance of getting an infective feed of malaria Even if it should chance that some of them get an infective feed, the chances of transmission of the parasites to a human being are very remote It is impossible therefore to conceive of any jungle anophelines playing an appreciable part in the epidemiology of malaria

The possibility of a reservoir of infection of human malaria existing in wild animals and serving to infect the jungle mosquitoes has been an obsession of the lay mind It is often thought on the analogy of the reservoir of infection of trypanosomes in wild animal in Africa, that it is possible that wild animals in the jungle could similarly act as reservoirs from which jungle mosquitoes could get infected with malaria and transmit the infection to human beings living inside or close to jungle Workers have from time to time attempted to ascertain if animals living inside forests harbour the human malaria parasites All these attempts to ascertain if the human malaria parasites exist elsewhere than in man have been entirely negative In India, Donovan (1920) investigated the blood parasites of monkeys, bats, squirrels and other animals of the forests in the submontane zone of the Nilgiri Hills and reported that the human malaria parasites are not found in any of the various animals that he investigated There is little doubt at the present time that the three species of parasites causing malaria in man are entirely human parasites

The investigations recorded in the present paper show not only that species of *Anopheles* which occur inside jungle in Bengal are harmless to man, but that jungle forms a definite protection against the breeding of species capable of transmitting malarial infection It would be harmful therefore to effect any extensive deforestation without taking proper safeguards by way of *Anopheles* control as by the opening up of forest land, breeding places which have been harmless hitherto are rendered favourable for the breeding of carrier anophelines These observations point to a preservation of all existing jungle and to extension of jungle wherever feasible

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THE ULTRA-VIOLET ABSORPTION SPECTRA OF SERA IN TROPICAL DISEASES

KALA-AZAR

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THE absorption spectra of blood sera in the ultra-violet region was first studied in a systematic way by Judd Lewis (*Proc. Roy. Soc.*, B 89, 1916, 327). From the many samples of normal sera examined he obtained results which were practically constant as regards the character of the absorption curves. He also examined a number of pathological sera some of which showed abnormalities. These observations led us to begin an investigation in connection with diseases prevalent in Bengal. We have made a beginning with kala-azar as already some peculiar physical and chemical changes have been noted on in this serum.

In our experiments we used a Hilger rotating disc sector photometer in conjunction with a Hilger quartz spectrograph and as a source of illumination a powerful condensed spark between carbon electrodes impregnated with uranium and molybdenum salts in order to produce a large number of lines throughout the spectrum specially in the ultra-violet region. The plates used were Ilford panchromatic (backed) and on each plate were taken twenty photographs in addition to the copper spectrum and a test band taken with the sector fully open and nothing intervening in either path of light.

The serum before examination was freed as far as possible from corpuscles by centrifuging until a clear pale yellow liquid was obtained. It was then diluted with (physiological) normal saline to produce a mixture of one part of serum in fifty. This solution was filled into a one cm. observation tube fitted with quartz ends. A second tube was filled with normal saline solution

only and used as a blank in one path of the spectrophotometer, so that the observations made with the tube of serum solution placed in the other path express the absorption of serum only

The absorption curve was plotted with extinction coefficients as ordinates and wave lengths as abscissæ The wave lengths at the points where two spectrographs in juxtaposition have equal intensities were obtained with the help of a travelling micrometer by comparing with the copper arc spectrum and calculating the readings of the micrometer on the basis of the law of dispersion of prisms

Several specimens of normal blood sera were examined with results which are very nearly constant The general character of the curve is also in close agreement with that recorded by Judd Lewis (*ibid*) We also examined many specimens of kala-azar sera kindly supplied to us by Dr Napier from the Tropical School Hospital, Calcutta The curves obtained from the latter were also almost constant and quite comparable as a group The experimental data are given in Tables I and II The values brought together in the tables for comparison are the wave length of the region of greatest absorption in the band, i e, at the head, the wave length at the point of least absorption in the band, i e, at the foot of the curve in the depression, the extinction coefficients (log $1/I_0$) ε_h and ε_f at the head and foot respectively of the absorption band, and the ratio $\varepsilon_h / \varepsilon_f$

The ratio $\frac{\text{Extinction coefficient at head}}{\text{Extinction coefficient at foot}}$, which is according to F C Smith (*Proc Roy Soc*, B 104, 1929, 198) an index of purity of a substance, may we think, be taken also as an index of the constancy of composition of the sera under investigation

TABLE I

Normal blood sera

Specimen No	Wave length		Extinction coefficient		$\varepsilon_h / \varepsilon_f$
	Head	Foot	Head ε_h	Foot ε_f	
49	2,780	2,540	0 85	0·45	1 89
50	2,785	2,535	0·87	0·45	1·89
51	2,760	2,540	0·85	0·45	1 89
52	2,780	2,530	0·80	0 42	1 9
53	2,770	2,540	0·85	0·45	1 89
54	2,780	2,535	0·80	0·45	1·79
Adopted	2,780	2,540	0·85	0·45	1·89

TABLE II

Kala-azai sera

Specimen No	WAVE LENGTH		EXTINCTION COEFFICIENT		$\varepsilon_h / \varepsilon_f$
	Head	Foot	Head ε_h	Foot ε_f	
59	2,790	2,510	1 10	0 50	2 2
60	2,800	2,520	1 05	0 50	2 1
61	2,800	2 530	1 05	0·47	2·2
62	2,790	2,520	1 10	0 50	2·2
63	2,785	2,520	1 15	0·55	2·1
64	2,797	2,515	1 10	0 50	2·2
Adopted	2,800	2,520	1 10	0 50	2·2

In the table for normal sera it will be noted that the figures exhibit a remarkable regularity in the ratio $\varepsilon_h / \varepsilon_f$ showing the constancy of composition of the different samples examined The same is also exhibited by the figures for kala-azai serum The extinction coefficients of normal sera contrast strongly with those for kala-azai sera, the ratio $\varepsilon_h / \varepsilon_f$ for normal sera is only 1 89 while that for kala-azai sera is 2 2 The distinction is not in this ratio only, the extinction coefficients at the head and foot of the kala-azai sera are fairly high as compared to the normal sera, so that one is markedly differentiated from the other Again the wave lengths at the head and the foot of the curve though nearly the same are yet clearly not identical in both cases Still it is mainly in the magnitude of the extinction coefficients at the head and the foot of the curve that the difference between these sera find expression

Two absorption curves (Figs 1 and 2) have been drawn one for normal and the other for kala-azai serum with values which are deemed to be the nearest to the actual values The close semblance in form between the two curves would ordinarily signify a difference in concentration only of the serum but in view of the fact that there is a small but substantial difference in the ratio $\varepsilon_h / \varepsilon_f$ a further explanation should be sought In all probability the explanation for this difference lies either in some change in the chemical nature of the protein fraction of the serum or in some change in the relative concentrations of the constituent proteins as this fraction (Judd Lewis,

Proc Roy Soc, B 1922, 178 and F C Smith, *Proc Roy Soc*, B 104, 1929, 198) is mainly responsible for the characteristic absorption bands of blood serum Also it is as well to consider the facts noted by F C Smith (*Biochem Jour*, 22, 1928, 1499) that the serum ultra filtrate has also its characteristic absorption band and that the absorption curve of the ultra filtrate of a pathological serum was found to deviate from the normal

Apart from the theoretical side of the phenomenon we were particularly interested in the suggestion made by Judd Lewis that this method might have a clinical application of practical value Unfortunately the method of calculating out the actual wave lengths of the different points in the curve is laborious and tedious To simplify this we have projected the negative of the spectrograph using an enlarger on to a sheet of white paper and marked out on this screen the points where any pair of spectrographs have equal intensities This is a comparatively simple and rapid process as the enlargement comes out in good prospective

In the cases of kala-azar sera that we have studied the difference from the normal is so well brought out that it is apparent to any casual observer We have given two such plottings (Fig 3) taken from the normal and the kala-azar sera against extinction coefficients and a wave length scale The originals were taken from the spectrophotographs of the two sera successively on the same piece of paper, the enlargement through the enlarger being equal in both cases and were subsequently reduced for convenience in reproduction to about one-fifth the actual size by photographing these after marking out the points with Chinese ink

We have also given two actual spectrophotographs with the points of matching marked on them (Plate XXV, figs 1 and 2)

We are indebted to the Indian Research Fund Association for supplying us with funds and to the Department of Physics, Presidency College, Calcutta, for allowing us to use their travelling micrometer for measuring wave lengths

Summary and conclusions

The absorption curve for normal blood serum has been found to be almost identical with that obtained by Judd Lewis

The absorption curve for kala-azar blood serum is distinctly different from that for normal serum

This variation is due not to a difference in the concentration only of the total protein of the serum, but probably to some change in the chemical nature of the proteins or to some change in the relative amounts of the constituent proteins

A simple and rapid method for comparing two spectrophotographs consists in projecting the plates on to a white paper and marking out the points of matching

PLATE XXV

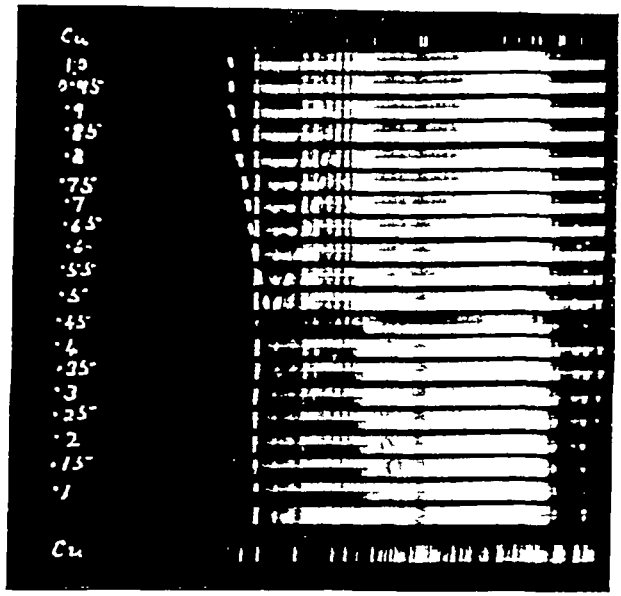

Fig 1 Normal Blood Serum

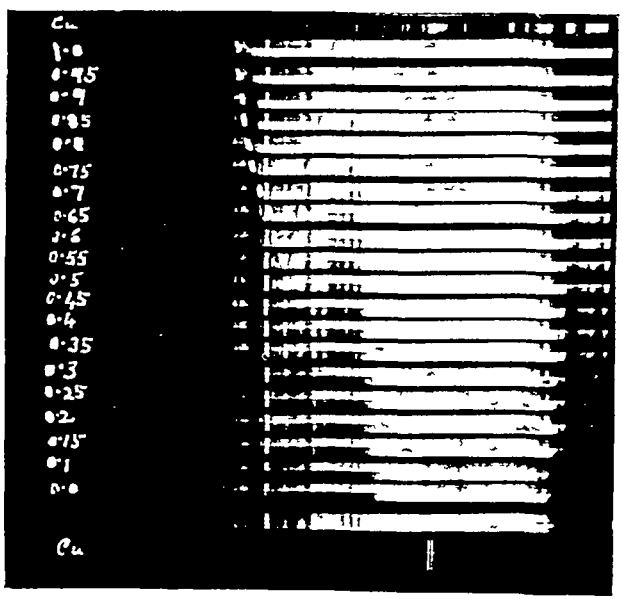

Fig 2 Kala-azar Blood Serum

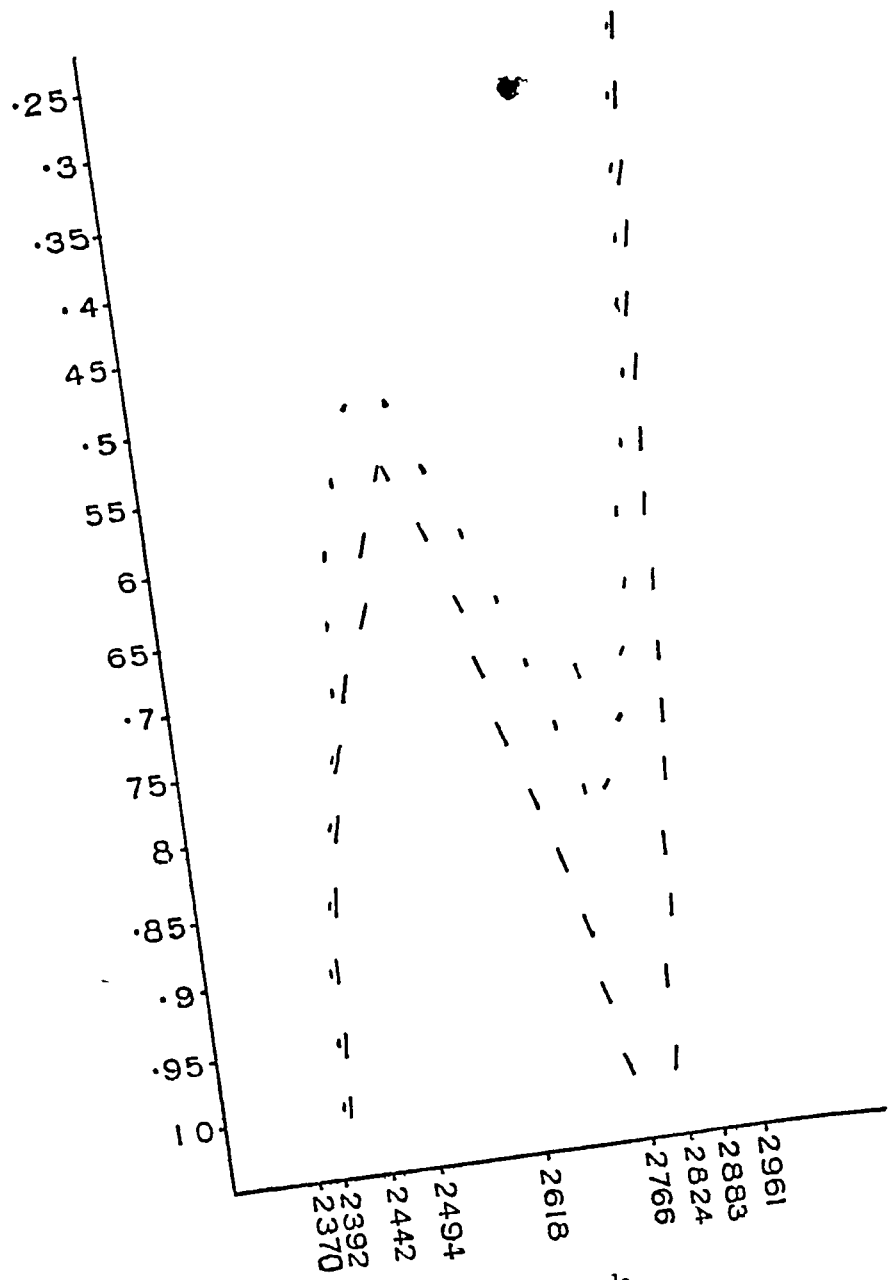
·25
·3
·35
·4
45
·5
55
6
65
·7
75
8
·85
·9
·95
1·0
2370
2392
2442
2494
2618
2766
2824
2883
2961
Wave length scale
--------Normal Blood Serum
— — — —Kala-azar Blood Serum

Fig 3

THE RÔLE OF URINARY COLLOIDS IN THE PREVENTION OF STONE FORMATION

BY

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SOME of the constituents of the urine are present in solution in greater concentrations than can be obtained in pure water and it is generally supposed that some special mechanism is necessary for the prevention of their precipitation. The mechanism is commonly supposed to be the urinary colloids, which either by adsorption to the insoluble crystalloid or by the adsorption of the crystalloids to them, prevent visible precipitation. The urinary colloids are supposed to be the chief—or one of the chief—factors in the formation of urinary stones. The arguments are well put by Joly, 'Stone and Calculous Disease of the Urinary Organs' (1929), London:—

'*The solubility of the urinary crystalloids*.—It is well known that the stone-forming salts present in the urine are all sparingly soluble. We must, therefore, inquire whether they are in a state of simple solution or whether some special mechanism is necessary to prevent their precipitation.

If we look at the question from the point of view of pure chemistry, the first point that arrests our attention is that normal urine is always a strongly supersaturated solution. For example, 0·56 mg. of anhydrous calcium oxalate, containing 0·351 mg. of oxalic acid, can be dissolved in 100 c.c. of water. This corresponds to 5·265 mg. in 1,500 c.c., yet the daily output of oxalic acid in the urine is from 15 to 20 mg. Normal urine therefore contains this substance in a strength which is from three to four times the maximum concentration obtainable in pure water. In pathological conditions this proportion can be greatly exceeded. Umber (quoted by Lichtwitz) recorded a case of oxalic acid poisoning in which the urine held in solution sixty-eight times as much calcium oxalate as could be dissolved in an equal quantity of water. The same applies

to uric acid At a temperature of 37°C 1 g of uric acid is soluble in 25 litres of water, yet approximately this amount is excreted in the urine every day The solubility of uric acid in urine is from ten to twenty times greater than in pure water

These sparingly soluble urinary salts do not therefore obey the ordinary laws of chemical solutions, and we must look elsewhere for an explanation of their abnormal solubility It depends on the presence of certain colloids in the urine This can easily be shown by dialysis If a specimen of urine is dialysed through the membrane of ordinary parchment paper against a large quantity of water, the crystalloids will diffuse through the membrane, while the colloids will be left behind If this solution of the urinary crystalloids is slowly evaporated down, it will be found that a copious deposit forms long before the solution is concentrated to the original volume of the urine This deposit consists of a mixture of uric acid, calcium oxalate and calcium phosphate Again, it is impossible to make an "artificial urine" by mixing together the various urinary crystalloids in the proportions in which they are found in normal urine Such a mixture always throws down a deposit The abnormal solubility of the stone-forming salts is therefore a consequence of the presence of certain colloids in the urine'

The argument that because certain slight soluble constituents of the urine dissolve more readily in urine than in pure water, seems to be a poor reason for supposing that the urinary colloids are the cause Urine contains many other substances besides the colloids which may effect the solubility Uric acid, for example, is well known to dissolve more readily in the presence of many substances, amongst others, phosphates, which are always present in urine Further, uric acid in the urine does not exist entirely as uric acid It is undoubtedly partly in the form of salts, and these presumably partly ionized, and perhaps some of it is in other unknown combinations The same applies to calcium oxalate—or rather since calcium ion is undoubtedly present in urine in large excess—to the oxalate ion

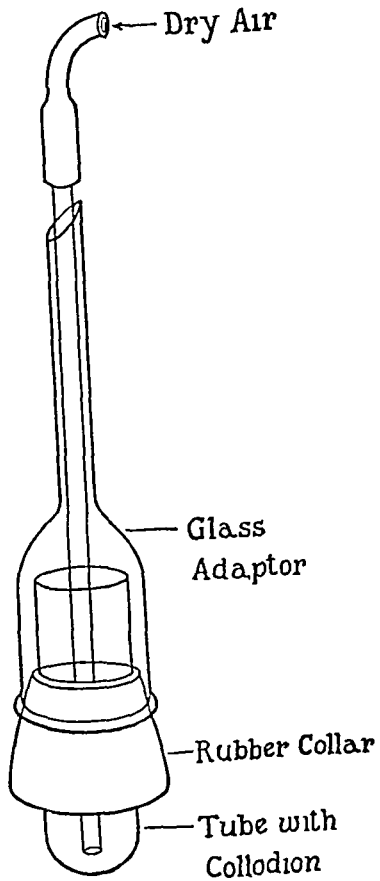
The arguments, on the other hand, from the results of dialysing urine are cogent and fairly convincing The experiments described in this paper were designed to test them

A number of experiments were made to determine the effect of various colloids (soluble starch gelatin, serum albumen and milk) on the solubilities of uric acid, calcium oxalate and earthy phosphates in an artificial mixture of salts, of similar composition to urine These experiments are not given in detail as they are open to the objections (1) that the colloids used were not urinary colloids, and (2) that the mixture although similar to urine, differed from urine in many respects besides the absence of colloids The results of these experiments were that of the colloids tried none had any influence on the solubilities of any of these three substances

A series of experiments were made with different samples of normal urine to determine how the uric acid distributed itself on dialysis between solutions

inside and outside the dialysis sac If uric acid, as an example of an insoluble urinary constituent, is held in solution by the presence of colloids, presumably, it must be either adsorbed on the colloid particles, or the colloid must wrap itself round the molecules or particles of uric acid, and in either case one would expect its diffusibility to be done away with or diminished

For these experiments collodion sacs of about 20 c c capacity were used They were made from ordinary collodion and considerable difficulty was experienced in getting sacs which were strictly semi-permeable, i e , let through crystalloids and at the same time prevented the passage of colloids The permeability as is well known depends on the amount of drying and with this collodion it was found necessary to dry the sacs for over an hour in a stream of specially dried air A small apparatus (*vide* sketch) was devised to do



this and when it was used the sacs for the most part turned out satisfactory The sacs were supported by being tied on to pieces of large bore glass tube covered with rubber and were suspended in small beakers of such a size that 15 c c of liquid inside the sac rose to the same level as 15 c c of liquid outside Before and after each experiment the sacs were tested by dialysing 15 c c of

a mixture of soluble starch and sodium chloride solutions against 15 c c of water, for 24 hours A sac was considered satisfactory if at the end of that time no starch was found in the dialysate and the concentration of chloride was the same (within 1 per cent) in both the outside and inside solutions

The results of five experiments, each on a different normal urine, are shown in Table I In each experiment 15 c c of urine were dialysed against 15 c c of water—a pinch of thymol being added with the intention of hindering bacterial decomposition, though from the rise in pH in all the experiments some decomposition must have occurred After dialysis the concentrations of uric acid outside and inside the sac were determined by Folin and Denis' colorimetric method In each experiment the concentrations of uric acid were the same (within the experimental error) within and without the sac, indicating that adsorption if any between the uric acid and colloids in normal urine is of the lowest kind The experiments lend no support to the view that uric acid is held in solution by the colloids

TABLE I

Showing distribution of uric acid inside and outside the sac on the dialysis of urine

Duration in hours	Inside	Outside	pH		Proportions of uric acid	
			Before dialysis	After dialysis	Inside	Outside
20	15 c c of urine + thymol	15 c c water	5·5	6·4	48	52
18			5·9	6·8	52	48
44			5·5	6·9	49	51
44			5·5	7·0	49	51
44			5·5	6·8	50	50
			Mean		50	50

The most conclusive of all these experiments were a series in which urine was dialysed against water In each experiment 20 c c of urine were dialysed for 24 hours against 20 c c of water By this means the colloids were confined to the solution inside the dialysis sac while the crystalloids distributed themselves about equally between the inside and outside solutions After the dialysis both the inside and outside solutions were carefully evaporated on a water-bath to 10 c c each, so that the crystalloids in each of the parts were now at the same concentration as in the original urine, while all the colloids of the original urine remained in the inside part. The pH was taken at each

stage of the process When the evaporation was complete note was taken of any precipitation which had occurred, and in the event of precipitation having occurred in the outside solution (urine free from colloids) the change in pH required to clear the solution was determined

In these experiments Schleicher and Schull's dialysis thimbles, of about 40 c c capacity, were used The thimbles were supported by being tied on to corks and were suspended in small beakers of such a size that 20 c c of liquid inside rose to the same level as 20 c c of liquid outside

Each sac was tested before and after each experiment for its non-permeability to soluble starch and for its complete permeability in 24 hours to chloride ion When the sacs were not in use they were soaked in dilute sulphuric acid to prevent the growth of moulds, and before use they were washed thoroughly with dilute ammonia and water till neutral (except in Experiments III and VIII)

At the conclusion of the dialysis the inside and outside solutions were transferred to test tubes graduated at 10 c c and evaporated in these In some of the experiments in addition to the two parts into which the urine was separated by dialysis another test tube was put up containing 10 c c of undialysed urine and 10 c c of water and this mixture evaporated along with the rest The results of nine experiments are shown in Table II

TABLE II

Summary of experiments on the dialysis of urine

Experiment	pH of original urine	Inside	pH	Outside	pH	Undialysed urine + water	pH
I	5 6	Cloudy	6 1	Clear	6·2	Cloudy	5 9
II	5 6	Cloudy	6 1	Clear	6 2		
III	5 6	Cloudy	5 2	Clear	5·3	Clear	5 6
IV	5 6	Clear	5 2	Clear	5·3		
V	5 3	Cloudy	7 4	Clear	7 4		
VI	5 3	Cloudy	7 4	Clear	7 4		
VII	5 5	Cloudy	6·8	Cloudy	6 8		
VIII	6 8	Clear	5 2	Clear	5 2	Precipitate	6·8
IX	5 4	Clear	6 8	Clear	6 8	Clear	6 8

The same urine was used for those experiments bracketed together, viz, I and II, III and IV, and V and VI In Experiments III, IV and VIII the sulphuric acid in which the sacs had been soaked was not entirely washed out

and the increase in acidity after dialysis is due to this In Experiments V and VI, on the other hand, the sacs were rinsed with dilute ammonia and the diminution in acidity after dialysis in these experiments is partly, if not entirely, due to this In Experiment IV, after evaporation, the chlorides were determined in the inside and outside solutions, to make sure that the time allowed was sufficient for the diffusible ions to distribute themselves evenly, and the ratio of their concentrations were found to be 51 to 49 In Experiment VII, in which both inside and outside solutions were cloudy after evaporation, with a pH of 7 4, this cloudiness was completely cleared up by the addition of dilute acid till the pH came to 5 5—its value in the original urine In Experiment VIII, in which an originally very slightly acid urine (pH 6 8) was made distinctly acid (pH 5 2) before dialysis, the resulting solutions still remained clear on adding alkali till a pH of 6 8 was reached but became cloudy on increasing the pH to 7 4 The undialysed urine used in this experiment gave a precipitate of phosphates on heating and required acid till its pH reached 5 4 before this cleared up In Experiment IX the change in pH from 5 4 in the original urine to 6 8 after evaporation was not due to any added alkali but was due to the heating The urine itself either alone or diluted with water showed the same change after heating to 95°C for half an hour

It will be noticed that precipitation occurred more often in the inside liquid (containing the colloids) than in the outside Where precipitation did occur in the outside liquid the solution was completely cleared by restoring the pH to its value in the original urine The behaviour of both the inside and outside solutions as regards precipitation seemed in each experiment to run exactly parallel and to be dependent only on the pH and have nothing to do with the presence or absence of the urinary colloids

Summary and conclusions

Against the arguments for the theory that the urinary colloids are the mechanism by which certain difficultly soluble constituents of the urine are prevented from precipitating, the experiments described in this paper have shown —

1 That no evidence was got that other colloids (starch, gelatin, serum, albumen and milk) had any effect on the solubility of such constituents (uric acid, oxalates and phosphates) in an artificial mixture of salts resembling urine

2 That on the dialysis of normal urine the uric acid distributed itself evenly on both sides of the dialysis membrane This shows that the uric acid is not firmly attached to the colloids

3 That if normal urine was dialysed and the dialysate evaporated to the concentration of the original urine no precipitation occurred if the acidity was kept at, or restored to, its original value A clear solution of all the crystalloids

(including the uric acid, oxalates and phosphates) in their original concentrations could be got in the absence of colloids, so long as the acidity was kept the same

Normal urine undoubtedly contains colloids, but it is very doubtful if these have any considerable influence on the precipitation of such constituents as ordinarily go to form urinary calculi

STUDIES IN PERNICIOUS ANÆMIA OF PREGNANCY

Part II

A SURVEY OF DIETETIC AND HYGIENIC CONDITIONS OF WOMEN IN BOMBAY

BY

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Introduction

A preliminary study of the diets of patients suffering from pernicious anæmia of pregnancy suggested that certain dietetic faults might be causatively related to this disease With a view to investigating this possibility further a large scale dietetic and hygienic survey of different classes of women in the city was carried out and the results will be presented in this paper

Classes of women investigated

Women of the childbearing age only were investigated The following classes were surveyed

A *Healthy Hindu women* of the middle and professional classes —These were studied as a control for the hospital class of women

B *Women of the hospital class* belonging to all the different communities in the city This class was subdivided as follows —

Group I —70 healthy women recently delivered of healthy, living children

Group II —25 women recently delivered of premature children but otherwise apparently healthy

Cases with any other abnormality, e g , severe anæmia, were excluded as we wished to investigate only those cases in which the cause of premature birth was unknown

Group III—20 *Hindu mill-workers*—These women are only representative of this one class of mill-worker and generalizations as to mill-workers as a whole cannot be made from the observations obtained all statements refer strictly to this one class of worker

Group IV—40 *old cases of 'pernicious anæmia of pregnancy'*—These cases had been discharged improved from hospital and at the time of observation were in sufficiently good health to pursue their normal occupations, chiefly housework

Among the hospital class studied there were women belonging to the Hindu, Mohammedan, Christian (Goanese), Bene-Israel and Parsee communities Both meat-eaters and vegetarians were, therefore included in this class With the exception of some of the mill-workers and a few women who were married to men who were out of work the women were not of the poorest classes and in fact many were wives of well-to-do shop-keepers and tradesmen and were comfortably off

Blood counts

The members of Class A (better class normals) have an average red cell count of 4 885 millions per c mm and an average white cell count of 4,890 per c mm The hæmoglobin estimations in this class were faulty and have been excluded from the series The average red cell count, 3 86 millions of all the hospital groups together (Class B), is significantly lower than that of the better class controls Considering each group separately and comparing with the hospital controls (Group I) (Tables III and IV) it is seen that there is no significant difference between the mean red cell counts of Group I and II (controls and prematures) but that in Groups III and IV (mill-workers and old anæmias) there is a significant lowering The hæmoglobin values of the four groups were 9 6, 9 5, 8 61 and 8 1 grammes per cent respectively Major Sokhey in a personal communication has given us the figures for his series of normal Indian women and in this paper these values are used as standards His standard values are 4 5 millions red blood cells per c mm and 12 99 grammes hæmoglobin per cent Using 5 00 millions as the standard blood count for convenience and 12 99 as equivalent to 100 per cent hæmoglobin, the colour indices of the four hospital groups are 0 92, 0 945, 0 89 and 1 04 respectively These figures indicate that with the possible exception of the mill-workers the counts and hæmoglobin values are reduced proportionally The white cell counts (Tables III and IV) show no very definite change but are low normal values These low values are very constant and in the hospital control group the range is very small, further work is needed to elucidate the significance of these findings

I Dietetic survey

1 *Method**—The method of survey is as follows —(1) With the exception of better class control cases who weigh and record the food eaten at every meal for 5 days, the women are visited in their homes on five consecutive days

* Elaborated with the help of Colonel McCarrison

This is essential when dealing with women of the hospital class in this country as they are incapable of giving an accurate account of what they eat and drink

(2) A careful record is made of all food bought and as a check a note of what the woman actually ate herself during the preceding 24 hours The greatest patience is needed and leading questions should be avoided Food bought by the month must be noted as well as the daily marketings The actual weight of food bought is recorded, and when articles of diet are bought by volume the weight of the standard volume of each food-stuff so bought must be determined It is essential to get the confidence of the household, or else inaccurate information will be given, and absurd figures obtained

(3) The amount eaten daily is calculated as follows —(*a*) Individual items such as milk or special food eaten by the women only are recorded with quantities consumed (*b*) Items shared by the family are assumed to be eaten in the following proportions (Lusk's coefficients) male over 14, 1 00, female over 14, 0 83, child 10 to 14, 0 83, child 6 to 10, 0 70, and children under 6, 0 50 The number of persons sharing the food must be known and it is essential to remember that individuals other than the immediate family frequently share Food bought daily is measured in this proportion but for articles bought by the month the monthly amount is divided in the above proportions and the daily consumption calculated The family's combined income should be used as check on the calculated food consumption The daily average intake is the mean of the total intake for the 5 days

The daily intake of different items of food being known it is easy to calculate from published tables (Schall and Heisler, 1917 and McCarrison, 1928) the proportion of carbohydrate, protein and fat consumed, the proportion of animal and vegetable products and the calories used

In addition to the above some method of assessing the intake of vitamins A, B and C is wanted, vitamin D is assumed to be obtained largely from exposure to sunlight For this purpose purely arbitrary figures were given to each common food-stuff to express their relative richness in vitamin content, and the total richness in vitamins of each diet calculated by the addition of these figures This method is purely arbitrary but as the food-stuffs are, with certain exceptions to be noted later, common to all the groups, it affords a fair means of contrasting the vitamin content of the different diets Table I gives the figures used Those for milk will probably be criticized as too low but the values are purposely given as in Bombay the milk is very adulterated and in addition all classes boil the milk for long periods The milk used by the better class women is assumed to be less adulterated than the ordinary bazaar milk The arbitrary value given for pure ghee is intentionally comparatively low, but the antimony trichloride test even in good samples suggests that the vitamin A content is low either because of the method of preparation or because of the condition of the cattle In Tables III and IV it will be seen that there are two columns for vitamin A The first column is calculated on the assumption

TABLE I

Arbitrary values to indicate relative richness in 'vitamin content of 1 oz food-stuff'

	A	B	C
Milk *	5·0 Better class controls 2·5 All other classes	1·0 0·5	0·0 0·0
Ghee and butter	20·0		
Atta, Bajree	2·5	10·0	
Polished rice			
Patni		5·0	
Dhal and gram	3·0	15·0	
Roots other than carrots		5·0	10·0
Greens and tomatoes	5·0	5·0	20·0
Non-green vegetables		3·0	10·0
Oranges	3·0	3·0	25·0
Other fruits		3·0	10·0
Eggs	15·0	10·0	
Meat chicken, fish		1·0	
Liver	15·0	15·0	5·0
Coco-nut		7·5	

* Boiled Much adulterated

that all the ghee contains vitamin A, in the second, the figures are corrected for the actual percentage of ghee consumed that was found, by the colorimetric test, to contain vitamin A (*see* Table II) The better class women generally

TABLE II

Presence or absence of vitamin A in samples of ghee as judged by the colorimetric test

Class of case	No of samples	PRESENT		ABSENT	
		No	Percentage	No	Percentage
A Better class controls	16	13	80	3	20
B I Hospital controls	42	21	50	21	50
II Mothers of premature babies	14	7	50	7	50
IV Cases of anæmia	15	4	26	11	74

made their own ghee and 80 per cent of the samples tested gave a positive reaction with the colorimetric test, whereas the ghee used by the hospital classes is grossly adulterated The figures given are on the side of generosity as any ghee that gave the slightest colour reaction was given full marks for vitamin A

Standard adopted

The results of the survey of Class A (healthy Hindu women) are taken as normal standards, and the other groups (Class B) contrasted with these In addition the results obtained from the normal hospital cases are taken as a normal standard for the hospital class of women, as it is the differences between the different groups in this class that are important in determining the dietetic faults that may underlie the different clinical entities studied

Results

1 *The Nature of the food consumed*—*See* Table V

Class A—*Better class controls*—Members of this group eat both lacto-vegetarian and mixed diets Milk and milk products figure adequately in the diet and are a good source of vitamin A Polished rice and wheat are the staple cereals, rice predominating The maximum daily intake is 7 0 ozs rice and 11 3 ozs wheat, but the average daily consumption per person is 4 ozs rice and 3 7 ozs wheat A small amount of dhal is usually taken daily The amounts of fruit and vegetables eaten is high insuring an adequate intake of mineral salts The mixed diets contained small quantities only of meat, fish and eggs, so that the bulk of the calories are obtained from carbohydrates and fat, of which the latter is eaten in large quantities

Class B—It can be said of all the groups in this class that the amounts of milk and milk products and of fruit and vegetables, and therefore of the mineral salts, in the diet are very low as compared with those of the better class controls Fats too, both animal and vegetable, are taken in much smaller quantities The main bulk of the calories are obtained from carbohydrate, more especially from the cereals of the diet There are striking differences too between the nature of the cereals eaten and the composition of the diets of the different groups in this class

Group I—*Normal hospital controls*—The diets eaten by members of this class contain a small and fairly constant supply of milk and ghee, both however of very inferior quality The fat intake, both animal and vegetable, is less than half that of the better class control cases Cereals are however eaten in much larger quantities, polished rice again forming the staple cereal with the addition in certain cases of small quantities of patni (whole rice), wheat—this grain sometimes in fair quantities,—white bread, and bajree in exceptional cases A small quantity of either dhal or gram is generally taken daily Sugar in fair quantities is eaten The supply of fresh fruit and vegetables is low The majority of the cases eat a small quantity of meat and fish, not generally daily, and sometimes an egg

TABLE III

Daily food consumption, blood count, housing and income of different classes of women in Bombay

		AVERAGE DAILY INTAKE PER PERSON IN GRAMMES					CALORIES	VITAMINS AVERAGE DAILY INTAKE IN ARBITRARY UNITS *				Red blood cells per c mm average count in millions	White blood cells per c mm average count	Cubic space (ft.) per person	HUSBANDS' INCOME PERCENTAGE		
		Total protein	Total protein	Total fat	Animal fat	Carbohydrate	Daily intake per person	A	Corrected A ‡	B	C				Under Rs 50	Rs 50 to 100	Over Rs 100
Better class controls	Mean	69	26	120	55	343	2,730	95	89	132	136	4·88	4,890	2,845			100
20 cases	σ †	24	19	42	24	108	658	40	30	52	32	0·388	1,126	2,270			
Hospital controls	Mean	54	17	46	18	308	1,860	31	27	83	47	4·06	3,837	524	51	35	14
70 cases	σ	18	10	21	14	93	656	16	12	43	26	0·384	572	345			
Mothers of premature babies	Mean	49	16	49	20	344	2,006	25	19	50	38	4·08	4,941	631	10	40	20
25 cases	σ	15	11	20	15	112	564	18	13	26	21	0·103	1,307	492			
Mill-workers	Mean	50	8	26	2	404	2,121	21	21	92	30	3·75	3,769	240	75	25	0
20 cases	σ	16	4	3	2	157	655	10	10	39	12	0·392	602	112			
Anæmias	Mean	46	11	45	20	294	1,758	31	24	80	33	3·44	5,169	550	37·5	42·5	20
40 cases	σ	14	7	20	14	76	471	19	16	36	17	0·813	1,360	328			

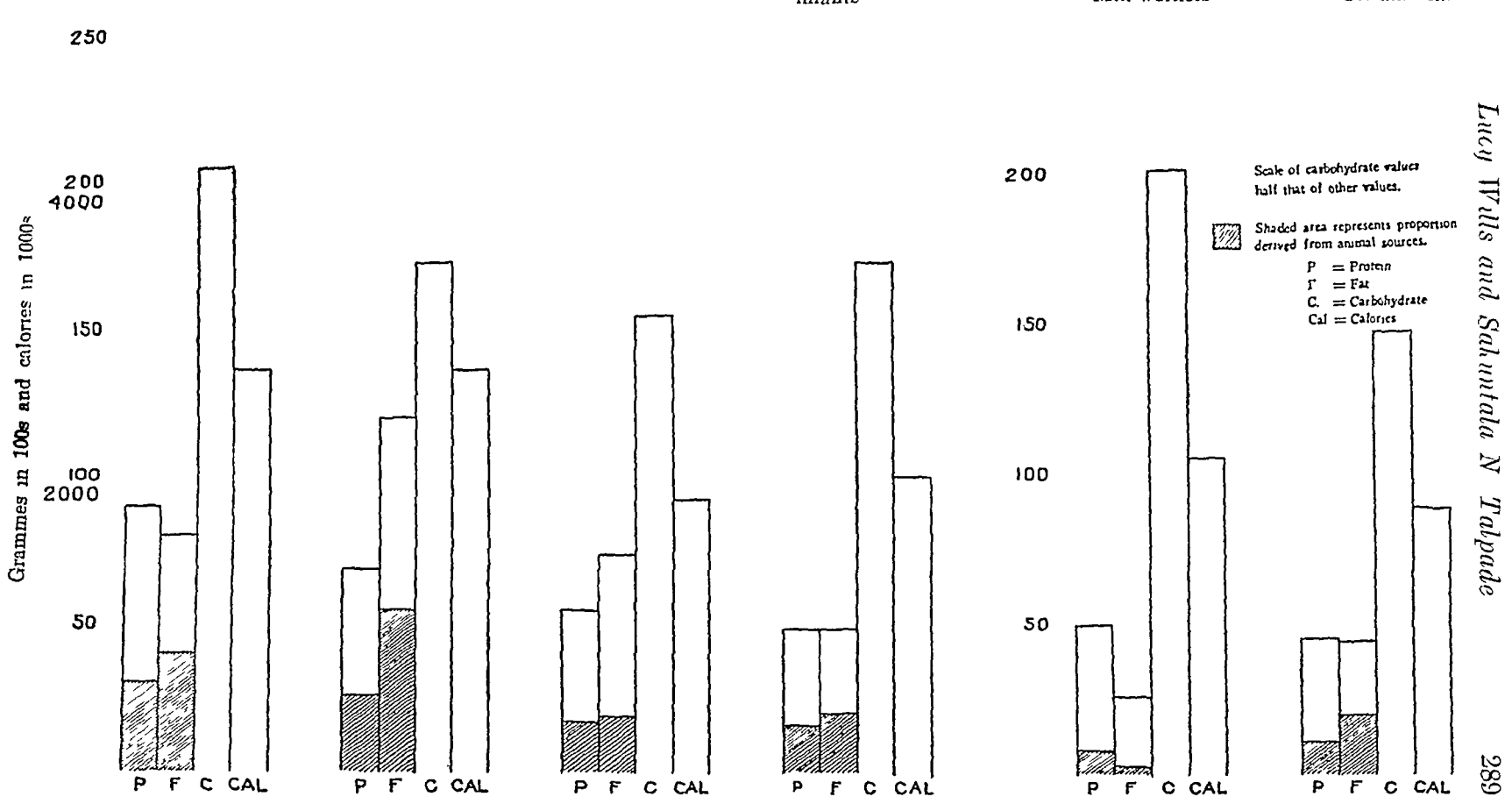
* *See* Table I

† Standard Deviation

‡ A units using corrected ghee figures *See* Table II

CHART

Proportion of protein, fat, and carbohydrate in diets and total calories



The diets of the members of the different communities do not differ very markedly but broadly speaking it can be stated that (1) the Hindus eat the better grains, (2) the Christians (Goanese chiefly) eat polished rice and white bread as their staple cereals, and these with the addition of a little meat and fish form the basis of their diet which is poor and ill balanced, (3) the Mohammedans eat less mixed cereals and more white bread and are generally meat eaters

In comparison with the hospital normals the next two groups show certain very distinct differences, whereas the findings in the other group (old anæmia cases) agree very closely with the hospital normals in the type of food eaten, though certain qualitative differences will be discussed in the next section

Group II—The diet of the women recently delivered of premature infants is very constant, the chief characteristic being the extremely poor quality of the cereals eaten, rice and white bread forming the staple articles of diet, whatever community the woman belongs to The next section will show that the actual calory intake and especially the proportion obtained from fat is higher than that of the control series but that the diet is very faulty because of the poor cereals eaten

Group III—The mill-workers' diet on the other hand is characterized by the very large cereal intake, which includes a very high percentage of bajree and a good deal of patni, so that the quality of the cereals is good compared with the other groups The intake of milk and ghee is negligible and the total fat intake very low Fish forms a constant item in the diet and about 50 per cent of the cases are meat eaters, but both these items are eaten in small quantities The intake of fruit and fresh vegetables is low

2 *Analysis of food consumed*

The diets eaten expressed in terms of carbohydrate, protein and fat, with the total calorie values and arbitrary vitamin figures are given in Table III Table IV shows the differences between the classes and the significance of the differences the upper figures in this table are the actual differences and the lower express the significance The differences are assumed to be significant when the lower figure is 3 or more and probably significant when over 2 This figure is obtained by dividing the difference of the means of the two groups by the square root of the sum of the squares of the standard errors

(*a*) *Comparison with better class controls* (*A*) —The most striking feature is the marked difference between the better class controls and all the other groups As compared with this class all the hospital groups are —

1 Under-caloried
2 Relatively low in both total and animal protein
3 Markedly deficient in both total and animal fat
4 Deficient in all 3 vitamins but especially in vitamins A and C

These differences (*see* Chart) must be considered in greater detail

TABLE IV

Differences between groups studied and the significances of the differences

	Total protein	Animal protein	Total fat	Animal fat	Carbo-hydrates	Calories	VITAMINS (Arbitrary units) Uncorrected A	Corrected A	B	C	Red blood count per c mm	White blood count per c.mm	Cubic space in feet
A *Better class controls used as a standard—*				In grammes									
Hospital controls	—14 *	—9	—74	—37	—35	—870	—64	—62	—49	—89	—0 820	—1,053	—1,321
(20)	2·4 †	2·0	7 5	6·6	1 3	5 1	6 9	8 0	3·8	10·9	8·0	1 4	2 6
Mothers of premature babies	—19	—10	—71	—35		—724	—70	—70	—82	—98	—0 809	+54	—2,214
(25)	3 0	2·1	6·8	5 6		3·9	7·2	8·7	6·3	11 4	7 0	0·1	4 4
Mill-workers	—19	—18	—94	—53	+61	—609	—74	—68	—40	—106	—1 132	—1,121	—2,605
(20)	2·9	4 1	10·0	9·8	1 4	2 9	8·0	8·6	2·6	13·2	9·0	3·9	5 2
Anæmias	—23 0	—15	—75	—37	—49	—972	—64	—65	—52	—103	—1 445	+279	—2,295
(40)	4 0	3 4	7 5	6·2	1 4	5 9	6·7	8·1	3·2	12 8	5 6	0·8	4 6
B *Hospital controls used as standard—*													
Mothers of premature babies	—5	—1	+3	+2	+36	+146	—6	—8	—33	—9	+0·011	+1,107	+107
(25)	1 3	0·4	0 6	0·6	1 4	1 0	1 5	2·6	4 3	1 7	0 1	1 5	1 0
Mill-workers	—4	—9	—20	—16	+96	+115	—10	—6	+9	—17	—0·312	—68	—284
(20)	0·9	6 0	7 7	9·4	2·6	0·7	3·3	2·1	0·8	4 0	3·0	1 0	5 8
Anæmias	—8	—6	—1	+2	—14	—248		—3	—3	—14	—0·625	+1,332	+26
(40)	2 6	3·7	0·2	0·7	0 9	2 3		0·8	0 4	3·3	2·5	1 8	0·3
C *Anæmias used as standard—*													
Mill-workers	+4	—3	—19	—18	+110	+363	—10	—3	+12	—3	+0 313	—1,400	—310
(20)	0·9	2·0	5 7	7 8	3·0	2 1	2·6	0 9	1 0	0·8	1 2	5 3	4 4

* Upper figure $= m_1 - m_2$, the difference of the means

† Lower figure $\frac{m_1 - m_2}{\sqrt{\Sigma_1^2 + \Sigma_2^2}}$, the difference of the means divided by the square root of the sum of the squares of standard errors

The difference is considered significant if this figure is 3 or more and probably significant if between 2 and 3

TABLE V

Diets of better class Hindu women Class A

No	Religion	Milk	Ghee and butter	Vegetable oil	Rice	Patna	Wheat	Bajree	White bread	Juari	Dhal	Gram	Sprouted gram	Sugar	Roots	Potato	Tomato	Greens	Other vegetables	Onions	Fruit	Coco-nut	Meat	Fish	Eggs	Goguv	Liver
1	H	5·0	1·9	1·0	7·0		11·3	4·9			2·5	0·4		1·9		1·5		1·0	8·1		1·4	0·6					
2	H	2·3	0·8	1·1	1·1		6·0					0·9		2·2		1·7		5·2	2·0	0·3	1·0	0·2				0·3	
3	H	8·2	1·8	1·8	7·2		6·2							2·9		1·7	0·4	2·0	3·5		0·6	0·7					
4	H	7·0	1·9	0·9	5·4			1·3			1·2	0·1	0·3	0·7	2·2	2·2		1·1	7·9			1·4	0·8	0·6	0·9		
5	H	8·7	1·2	1·4	6·8		3·1	0·6	0·2		1·6	0·9	0·2	1·7		4·2		2·8	10·5	2·1	0·8	0·9	2·0	2·8	0·2		
6	H	8·3	2·2	1·0	5·7		6·0				3·0	0·7		3·3		3·6			3·7	3·1	2·0	1·2	1·2	3·3	1·1		
7	H	8·3	2·2	1·0	5·7						3·0	0·8		3·3		3·6	0·7	1·3	1·7	2·0	2·4	1·0	5·2	1·7	0·4		
8	H	4·4	0·9	3·0	3·4		2·0				0·2			3·8		3·7		1·4	6·6	3·1	1·0	0·9	3·3	4·3			
9	H	4·4	0·9	3·0	2·8		1·7				0·2			3·8		4·8			6·5	1·8	2·9	0·6	3·2	5·1			
10	H	7·0	1·9	0·9	3·3		4·0				2·0	0·6		0·9		1·6		2·7	3·1		5·1	0·2				0·2	
11	H	4·8	1·3		4·9		4·5				1·1	0·4		0·8		2·3		1·0	4·6			0·5				0·2	
12	H	7·6	1·6	2·0	4·7		4·3	2·3			2·2	1·6		2·4	1·1	1·9	0·5	2·0	1·0		0·3						
13	H	7·3	1·2	0·7	3·8		1·3	0·1		0·3	0·8	0·5	0·1	1·9		1·1		2·2	3·3	1·4	2·8	0·9	1·0	1·5		0·2	
14	C	2·6	4·5	1·0	1·5	1·2	3·0		1·7		1·1	4·0				5·6		7·8	1·2	5·6	1·4	3·5	3·3	0·5	0·9		
15	H	3·5	0·5	0·9	3·3		1·8				0·9	0·1		1·4		0·5		2·3	2·3	0·5	3·4	2·0		3·1			
16	H	7·0	1·3	3·3	1·9		5·3				0·4			2·0		0·8	0·4	4·7	1·6	3·0	3·0	2·5	1·3	1·6	0·4		
17	H	9·9	1·3	0·8	2·9		3·6				2·0			2·0		0·8		2·0	0·8	0·8		0·6		0·4		0·7	
18	H	11·2	3·5	2·5	2·2		2·7				0·5			2·0		1·8	0·6	3·0	1·5	0·6	3·0	1·9	2·1	1·8		0·3	
19	H	10·5	1·5	1·9	3·4		3·1				1·5			1·5		1·8		3·7	5·5	0·9	1·0	1·4					
20	H	7·0	0·7	0·9	5·4		5·0	1·3										6·2	1·1		3·0	1·7					

(1) The low calorie value is due largely to the extremely low fat intake, in Group III (mill-workers), however, where the fat intake is very markedly reduced and the animal fat consumed negligible, the calory deficiency is partly compensated by the enormous amount of cereals eaten

(2) The total protein is significantly reduced in all classes except the hospital controls where it is only probably significantly reduced In both Group III (mill-workers) and Group IV (anæmias) the animal protein is also significantly reduced, especially in the former group where the mean daily consumption is only 8 grammes a day

(3) The fat deficiency in the hospital class is very marked, especially in the diet of the mill-workers In the other three groups though the fat, both total and animal, is very much reduced the reduction in the proportion of animal fat is not so extreme as in the diet of the mill-workers, where it is only 1|13th of the total fat instead of nearly $\frac{1}{2}$ in the better class controls and less than $\frac{1}{2}$ but greater than 1|3 in the remaining groups

(4) The vitamins A, B, and C with the exception of the vitamin B in the mill-workers group, are all present in very significantly decreased quantities The lower values are due largely to the small amount of milk and milk products and fruit and vegetables in the diet and to the replacement of unmilled grains by white bread and polished rice The milk and ghee are also grossly adulterated so that their A content is low and the method of preparation of the ghee and the custom of boiling the milk for a considerable period further reduces it Vitamin C is low because of the small quantity of fresh fruit and vegetables eaten The amount of B varies largely with the grains consumed and as the better-to-do classes tend to eat more and more polished rice and white bread, it is these classes that are deficient in this vitamin, whereas the mill-workers who eat large quantities of unmilled bajree (pearl millet) and a good deal of unpolished rice (patni) have an adequate supply of B in their diet though it is probably significantly lower than that of the better class controls

(*b*) *Comparison with hospital controls*

If the hospital groups are compared, using the hospital controls (Group BI) as a standard, certain differences are seen The total protein values in the four diets are not significantly different, though the mean intake in the old anæmias class is probably significantly lowered But the proportion of animal protein eaten varies markedly in the four groups The cases in Group II (prematures) eat practically the same amount of animal protein as the control group but the mill-workers' diet has only about half and the diet of the old anæmia cases only about three-quarters of the standard amount, so that the values in both these groups are very significantly lowered

The mean values for both total and animal fat consumed do not differ significantly from the standard values with the exception of those of the

mill-worker group In this group the fat consumption is amazingly low—the animal fat intake actually only averaging 2 grms a day

The calory values of the diets are not significantly different, though owing to the varying proportions of the three groups of food-stuffs, the calories are derived from different sources, in the case of the mill-hands very largely from carbohydrates

The differences in the vitamin values are also interesting As stated above, all the values are low in comparison with the better class normals but there are certain differences in the hospital groups that must be noticed Using *uncorrected A values the only group that shows a significant difference* in its vitamin A intake is the mill-worker group If, however, corrected values for A are used the values are altered, with the exception of those of the mill-worker group which does not depend on ghee for vitamin A As a result of these changes the value for this group is no longer significantly lowered relatively to the standard for corrected A, but the value for the premature group is probably significantly lower Using either set of vitamin A figures, there is no significant difference between the value for the hospital control group and that for Group IV (old anæmias) The position is reversed as regards vitamin B, the only group showing any significant difference is Group II (prematures) where the vitamin B in the diet is markedly reduced owing to the poor quality of the cereals eaten The diets of both the mill-workers and the old anæmic cases are significantly low in vitamin C as compared with the hospital control value

Considering the three vitamins together and using the hospital control values as standards, Group II (prematures) is absolutely deficient in B but not in A and C, Group III (mill-workers) is deficient in A and C but not B, and Group IV (old anæmias) is deficient in C but not in A or B Relative to normal standards all the hospital class are deficient in all three vitamins, with the possible exception of vitamin B in the mill-hand group If our theory is correct that it is a deficiency of vitamins A and C that underlies this condition, all the hospital class should be potential cases of anæmia of pregnancy, whereas this is not true of the mill-workers, who in spite of their poor diet are very free from this condition (Balfour, 1929) With a view to possibly getting a clue to this riddle the mill-worker group (III) is compared with the old anæmia cases group (IV) Using corrected values for vitamin A it is clear that there is no difference between the two classes It must be kept in mind, however, that the diet of the mill-workers is extremely low in fat, both animal and vegetable, in other words, that the diet of the old anæmia cases is relatively high in fat

II Hygienic survey

The hygienic conditions of the women depend largely on two factors, housing and exercise Both of these for economic reasons must depend on the means of the people An attempt was made to assess the incomes of the

households by inquiries as to the wages or income of the husband and if she worked, of the woman too The actual figures could only be obtained when the individuals were wage earners, and even then owing to the family system, it was frequently difficult to assess the total means for the people sharing the house and food For these reasons the figures are not given but the incomes of the different classes are grouped under 3 headings (1) under Rs 50 a month, (2) 50 to 100 rupees a month, and (3) over Rs 100 a month

The better class controls naturally fall into the third class In the four hospital groups the mill-workers are obviously the poorest, 75 per cent of the women investigated falling into the lowest class and none in the upper class The old anæmia cases were better off than either the controls or the mothers of premature babies, showing, as previously stated, that these cases do not come from the poorest strata of society

Housing—As a rough guide to the degree of overcrowding the cubic space in feet of the rooms the women studied lived in was estimated and this divided by the number of people sharing the room The figures given in Tables III and IV show at once the overcrowding of the hospital class which becomes extreme in the mill-worker group Here sometimes as many as 15 persons share a room roughly 14 × 12 × 10 for which a rent of Rs 15 is paid, or 6 persons may share a room 10 × 10 × 8 for which a rent of Rs 5 is paid

Exercise and Purdah—The only classes that take regular exercise are the better class controls and the mill-workers, who have the daily walk to and from the mills as well as their work in the mills It is probable that this exercise and the better hygienic conditions of the mills as compared with their overcrowded houses that in part accounts for the relatively good health of this community The women of the remaining hospital groups take little exercise The city has a good water supply laid on in many cases to the houses, the women do not grind their own corn or perform other outside duties as in their villages, so that the only exercise is the daily marketing and the little housework needed to keep their small establishments clean Purdah is only observed by the Mohammedans, a class of women in this city shown by Balfour (1929) to be peculiarly liable to anæmia of pregnancy and the toxæmias of pregnancy In this connection it should be noted that though taking little exercise the Mohammedan women as a whole have a high caloried diet and a relatively high fat consumption

The question of malaria and infectious and contagious diseases is outside the scope of this inquiry, but broadly speaking malaria is common to the whole city, whereas it is probable as in other cities that the infectious disease rate is higher in the more crowded areas

Discussion

From the data obtained in this inquiry, it is obvious that in striking contrast to the better class of women in the city, who are well-fed and on both European and Indian standards not anæmic, the hospital class of women

are anæmic and both quantitatively and qualitatively ill-fed As the classes of women studied are very diverse and include members of all the chief communities of the city and different strata of society it is probable that these two defects are causatively related The anæmia is a chronic one and quite distinct from the acute condition known as 'pernicious anæmia of pregnancy' or 'Bombay anæmia,' though it is possibly a predisposing factor

The better class Hindu women are, as stated above, well-fed, though their diet is perhaps not perfectly balanced, as they obtain too large a proportion of their calories from fat and not enough from carbohydrate (*see* Chart) The total protein intake too is a little low It is generally thought that about a third of the protein and half the fat of the diet should be derived from animal sources and in this respect the diet of the class under consideration is satisfactory Fresh fruit and vegetables also figure largely in the diet

The diet of the hospital class is, in contrast to the above, very faulty The most marked defects apart from the inadequate supply of calories, are (1) the low total and animal protein intake and the lowering of the proportion of the latter product, (2) the low fat intake and the large excess in the proportion of vegetable fats to total fat, (3) the inadequate consumption of fresh fruit and vegetables The better-to-do members of the hospital class and also the Goanese eat cereals of an inferior quality, polished rice and white bread forming staple articles of their diet These defects lead to an inadequate supply of all the vitamins and the mineral salts which is further increased by the fact that the milk and ghee consumed are very poor in quality, the bazaar ghees in at least 50 per cent of the samples tested being entirely devoid of vitamin A as judged by the antimony trichloride test The food budgets in the Labour Office (Shirras, 1923) report on working class families in Bombay show the same defects

On our theory that a lack of vitamins A and C* underlies the condition of 'Bombay' anæmia, all the hospital classes are potential cases of this disease, some additional strain such as pregnancy precipitating an attack This is broadly true of all classes with the exception of the mill-workers, who are particularly free from this disease (Balfour) It is difficult to explain this immunity, but a comparison of the diet of this group with those of the other hospital groups and especially that of the old anæmia cases, brings out certain very striking differences The mill-workers do not depend on milk products at all for their vitamin A and so as long as their cereal intake remains good they have a constant, if small, supply of this essential factor The other groups in contrast depend very largely on a ghee supply which is grossly adulterated, so that their vitamin A intake is always liable to fail, further, these classes are tending more and more to replace natural grains by polished rice and milled flour so that their supply from these sources is steadily

* Cases of adult scurvy and xerophthalmia are not uncommon among the poorer classes in Bombay Beri-beri is practically unknown in the city

decreasing The supply of vitamin A, therefore, in the mill groups is low but probably fairly constant in addition they have a better supply of vitamin B than the other groups due to the nature of the grains eaten This group also eats a diet very low in fat It has been shown experimentally that the higher the fat intake the greater the demand for vitamins A and particularly B for proper growth and development (McCarrison, 1919 and 1930, Ederer, 1925, Harris and Moore, 1928, Plimmer and co-workers, 1927) Further Weitbrecht (1922) states that animals on a basal diet free from vitamins die more quickly if fat is added to the diet and that the animals getting fat develop a severe aplastic anæmia It seems possible that these experiments may explain the relative immunity from 'pernicious anæmia of pregnancy' of the mill-hands and the high incidence among the Mohammedan women in the hospital population which as a class is generally deficient in vitamins A and C The proportion of fat and vitamin B in the diet may be the deciding factor in the liability to the disease, and when, as in the mill-hand group, the fat intake is very low and the vitamin B content relatively high, it may require a more marked A and C deficiency to produce the disease Animal experiments of our own to be reported later support this theory but we recognize that other factors at present obscure may be concerned in the immunity of the mill-workers, and in the causation of the disease

The diets of the mothers of premature infants are of considerable interest in that all but one of the 25 cases studied had a very low vitamin B intake due to the fact that they one and all used polished rice and white bread as their staple cereals This remarkably constant finding differentiates this class from all the others and is not without significance in a consideration of the causes of prematurity, especially as vitamin B is one of the principal factors concerned in normal growth

The hygienic conditions of the different classes vary chiefly in degree as far as housing is concerned—overcrowding being common throughout the city but more marked in the mill areas Though these and such factors as exercise, may influence the incidence of the disease under consideration, the relative immunity of the mill-workers who live under the worst hygienic conditions, and the well-fed who live under the best, would suggest that the faults so marked in the diet of the affected classes are of greater importance in the etiology of this anæmia

Summary

(1) Class A (healthy Hindu women) is well-fed and not anæmic

(2) Class B (hospital class of women) is relatively to Class A both quantitatively and qualitatively ill-fed and also anæmic

(3) The anæmia is slight and due to a parallel reduction of both the number of red cells and the percentage of hæmoglobin

(4) The diet of Class B is deficient in (*a*) calories, (*b*) both total and animal protein and fat, (*c*) all fresh fruit and vegetables and therefore in

Diets of normal women of the hospital class (Class B I

No	Religion	Milk	Ghee and butter	Vegetable oil	Rice	Patni	Wheat	Bajree	White bread	Juari	Dhal	Gram	Sprouted gram	Sugar	Roots	Potato	Tomato	Greens	Other vegetables	Onions	Fruit	Coco-nut	Meat	Fish	Eggs	Gogary	Liver
1	H	5·8	0·5	0·6	5·4		1·6		2·0		0·8	1·6		1·4	0·9	0·9		2·2		0·5	0·2	0·5	1·0	2·0			
2	C	0·4	0·2	0·9	5·3				3·5		0·2			1·0								1·2	1·8	0·9	0·2		
3	C	2·4	0·3	0·2	9·8									2·5		0·2			0·2			0·5	1·6	1·3			
4	C	1·6		0·4	9·8				0·6					1·7		2·0						1·9	3·6	1·0	0·5		
5	C	3·2	0·2	0·8	9·8				3·5					1·7		0·8					0·2	1·6		1·5	0·5		
6		Not possible																									
7	H	20·0	2·0	2·5	3·9		6·4				1·8	0·9		1·3		2·4			4·7	0·7	3·0						
8	H	0·9	0·2	1·3	11·9		2·7	5·9	1·7		0·3	0·3		1·5		0·6			2·1	1·0		0·6	2·4				
9	C	1·3	1·1	0·1	10·5		1·5		0·7					0·9		0·9				0·4		0·8	3·3	0·3			
10	C	0·1	0·4	0·7	6·4				3·5					1·1		1·0				0·5		0·5	1·9	1·2			
11	C	4·1	0·1	0·5	12·7				3·5					2·2		0·6				1·1		1·8	3·3	1·6			
12	H	0·4	0·8	1·1	8·1	1·3		1·3			0·6	3·6		0·9	0·6	1·1		1·0	1·7	1·2		1·0	0·7				
13	H	3·2	0·3	1·3	10·0	4·8	4·5				1·1	1·1		0·8				2·0	1·0	1·3		1·6	2·2	5·1			
14	H	0·9	0·1	0·2	2·9	0·7	2·0	1·8	0·2		2·7			0·5		0·6		0·3	0·6	0·6		0·3	1·3	0·4			
15	H	0·9	0·1	0·2	5·7	1·3			0·2		0·3			1·0				0·3	1·5	1·9		0·7	0·8	0·3			
16	H	6·5	0·3	0·7	6·7	2·4					1·2	0·4		1·1				0·3	0·5	0·8		0·3					

17	H	44	03	07	51	08	23	08			23		08	17		16		03	17	08	16	01	06	21	02		
18	J	25	03	08	39		27	09	10		04	07		07				08	06	24		20	11	24	04		
19	H	46	06		72		33		12			08		12		30			06	03	14		25	11	05		
20	H	51	13	01	18		91				06	11		04		05		03	25	13	12	01	06		02		
21	H	05		04	57		13	57				05		11		12		10		09		06	13	03			
22	C	11	02		68		31		35		08			06		07			21	10		08	10	10			
23	M	07	01	02	41		28		10		19			03		09		13	42	02		03	24				
24	H	16	02	04	98	48		146			11	02		05		05		05	15	15		06	12	06			
25	M	26	18	11	80		22		17		22			09		08	01	03	16	11			34		11		
26			01	05	94	11		93	04		05	10		06		10		02	14	09		06	15	14	02		
27	Parsee	37	03	03	48				20		11			05		08	08	08	36	40	12		17	16	05		
28	M	09	02	05	41		25		14		25			05		06		06	15	07	06		14		02		
29	C	32	03	06	49		23					02		17		08		05		08		12	07	20			
30	C	44	05	12	102		23				02			22	04	02		07		04		07	10	10	07		
31	H	35	05	05	40		25			13	12			18		16	03	17	17	05		03	07	08			
32	M	22	01	04	34		47		03		27			05		10		04	18	09			15		02		
33	M	08	02	03	52		24		07		24	02		04		05	01	01	10	05		02	11				
34	M	04	23		34		11		21		11			12	28	35		07		12			30	02			
35	H	34	06	11	78		36				24			18	16	21		12		08		03	04	05	03		
36	M	16	03	10	98				35		06			08	10	10		10				16	29	25	02		
37	H	11	02	07	68		47				31			11		10		21	38		06						
38	C	11		08	71				17		16	02		12		04		04	04			04	10	16	03		

Diets of normal women of the hospital class Class B I—concld

No	Religion	Milk	Ghee and butter	Vegetable oil	Rice	Ragi	Wheat	Bajree	White bread	Juari	Dhal	Gram	Sprouted gram	Sugar	Roots	Potato	Tomato	Greens	Other vegetables	Onions	Fruit	Coco-nut	Meat	Fish	Eggs	Gogaru	Liver
39	H		06		98				16		04			25		10		10	25	08		18	15		01		
40	M	09	09	06	27		74		07		37			09		14		06	06	08			15		03		
41	H	16	07	10	92		09				21	02		10				05	38				07				
42	H	32	17		37		45				06	04		08		25	15	10	15	13	46		29	05	03		
43	Parsee	05	05	03	12				65		13			05		06			06	08			03	06			
44	H	33	06	07	25		47				16			06		21	03	07	21	07		09	20				
45	H			06	67			15			15	06				07		15	07	06		02	11				
46	H	07	01	02	44	21	20	22				11		08		07		10	15	06		03	07	05			
47	H	25	07	08	39		36				04			20		16		16	19	20			11	08			
48	C			13	49				32							10			10	05		13	29	18			
49																											
50	H	17		06	54		12				12	09		18		05		20	22			02	16	..			
51	C	16	02	02	86				70		04			13		02			10	05	30	09	14	15	02		
52	H	20	03	07	64		58				07			22		10		06	26	02			09	03			
53	C	19	03	01	60				29					15	06	09	06	03	15	06	38	10	31	18	04		
54	M	14	04	02	44		20				11	02		04		05		05	04	06				16			06

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Diets of mothers of premature infants Class B II

No	Religion	Milk	Ghee and butter	Vegetable oil	Rice	Patni	Wheat	Bajree	White bread	Juari	Dhal	Gram	Sprouted gram	Sugar	Roots	Potato	Tomato	Greens	Other vegetables	Onions	Fruit	Coco-nut	Meat	Fish	Eggs	Gogary	Layer
1	H	17	02	12	81		25		20			13		15		01		15		11	20	23	09	18	02		
2	C	21	01	10	43				35					06						02		05			13		
3	M	09	05	11	86		37				28	07		18		14	03			03	04		40				
4	H	08	19		25	30	11		20	25	06			06				01	18								
5	M	61	15	05	117	05	08		35			03		33		20			13			01	52				
6	C	11	07	01	80				35					07		04			12			05	20				
7	H	38	04	06	59		15		35		07	15		15	25	03			06		20	20					
8	H		01	03	34		39	08	20		08			09		07		01	35			03					
9	C			12	77				35					42		12		03	13			04	08	12			
10	H	25	08	10	24		33		35		07			15		23		03		01				07			
11	C			03	13				35					13								09		25			
12	C	25	08	03	155		12		35		17			20		12		07	17			07		28			
13	M	54	13	02	24		35				18	09		16		17		00		05		01	49	05	04		
14	M	18	01	12	77			160	07		10			13		02		02	08			01	07		01		
15	H	06	01	02	40	30			10		08			06	04	06			12	03		01	18				
16	H	11	01	02	140	05		05			26	02		14		03	06	04		03	06	04	09	12	02		
17	M	40	24	04	41		10		35		07			65		11	02			05		01	33				
18	M	19	02	02	22		14		63			01		06		06				03	06		11				
19	H	36	13	12	37	56	17				03	08		05		04		02	19	04	20						
20	H	05		01	127	08					02	06				03		15	26	09							
21	H		02	04	98		23				06	06		22		15		15	20				14				
22	M	20	05	07	128		31				08	03				13		10	20	10	30	02	14				
23	C	32	04	10	49				32					08		15	05	15	05	13	06	12	22	05	04		
24	H	63	16		74				35		06	23		17				10	35								

Diets of Hindu mill-workers Class B III

No	Religion	Milk	Ghee and butter	Vegetable oil	Rice	Patni	Wheat	Bajree	White bread	Juari	Dhal	Gram	Sprouted gram	Sugar	Roots	Potato	Tomato	Greens	Other vegetables	Onions	Fruit	Coco-nut	Meat	Fish	Eggs	Gogary	Liver
1	H	12		0·1	36			9·0			17	0·7		0·2				0·4	16	10				0·4			
2	H	19	0·1	0·2	11·9	2·8		2·9			10			10		12		0·6	0·9	11			0·4	12			
3	H	11		0·3	6·8	1·6		13·4	0·3		15			0·6		0·3		0·8	0·4	0·7		0·3	0·5	0·3			
4	H	10	0·1	0·5	4·8	3·0	0·7	3·0			15					10	0·2	0·3	10	0·5		0·1	0·9	0·3			
5	H	0·7		0·3	12·4	7·8					0·5	0·3		0·5				0·2	1·2	0·2				2·1			
6	H	12		0·3	7·7			7·7			0·4	0·7		0·5		0·4		0·4	0·4	0·4		0·1	0·6	0·8			
7	H			0·2	9·8				0·7		1·1	0·4				1·0		0·5	1·1					0·5			
8	H			0·3	7·7			7·7	0·4		0·9	0·8		0·4		0·8			1·6					0·8			
9	H			1·0	19·7	13·9					1·6			0·8		0·5		0·5	2·5	0·2		0·3	0·7	2·5			
10	H			0·7	9·5			9·4			1·4	0·3		0·4					1·6					1·3			
11	H			0·3	2·5			1·7	1·4			0·3						0·7	0·7	1·2				0·7			
12	H			0·6	7·1			7·1			0·6	0·3				0·4		0·7	3·0	0·5		0·9		0·7			
13	H			0·8	7·1			5·9	0·2		0·8			0·6				1·7		0·5		0·3	1·1	1·1			
14	H			0·7	6·4			3·1			1·4	0·3		0·4		0·4		1·2	0·6	0·4				0·7			
15	H			0·9	19·7						1·3	0·2				0·6		1·2	1·2	0·9				0·6			
16	H			0·5	6·0		5·6	5·9			0·8	0·5		0·4		0·3	0·2	0·1		0·2		0·1	0·4				
17	H			0·7	1·1		2·0	8·6			1·9			0·5		1·3		0·9	0·9	0·7			1·0	0·4			
18	H	1·1		0·9	2·1		1·3	8·5			0·2	0·4		0·5		0·6		0·6	0·6				0·4				
19	H	1·0		1·0	3·2		1·5	1·6			0·9					0·3		1·0	0·3	0·3		0·2	0·9	0·3			
20	H	2·2	0·1	1·2	6·8	3·2					0·6			1·2		1·0		0·7	1·4	0·4		0·4	1·0	0·4			

Diets of women who had suffered from anæmia of pregnancy Class B IV

No	Religion	Milk	Ghee and butter	Vegetable oil	Rice	Patni	Wheat	Bajree	White bread	Juari	Dhal	Gram	Sprouted gram	Sugar	Roots	Potato	Tomato	Greens	Other vegetables	Onions	Fruit	Coco-nut	Meat	Fish	Eggs	Gogiry	Liver
1	C	3·0	0·6		4·5				1·0		0·2			1·0	1·0			0·4			0·8	0·5	2·4	1·2			0·6
3	C	0·2			4·5				3·5					2·0							0·2	0·3		0·2			
4	H	2·3	0·4	0·8	3·6		1·7			2·5	0·6	1·0		1·3	0·5	0·7	0·2			0·2			0·8	1·4			
5	H	1·0	0·8	0·3	6·2	2·3	1·5			2·2	0·7	1·5		1·0	0·2	0·6				0·6	0·2			0·2			
6	H	2·1	0·3	0·7	4·4	3·3	2·0				1·0	1·0		1·5		1·1		1·5		1·2		0·7	0·5	1·2	1·0		
7	J	2·1	0·4	1·0	4·3	2·2	4·0				0·3	0·2		1·5		1·0	0·1	0·7	0·2	0·2	1·0	1·4	0·6	0·9			
8	J	1·8	0·3	0·6	2·7		2·5		0·9		0·3			1·4	0·3	0·8			1·1	0·1	0·1	0·2	1·2	1·1	0·7		
9	J	1·0	0·1	0·4	4·5		2·5		1·7		0·1			0·6		0·5		0·1			1·0	0·3		2·0	0·3		
10	M	7·0	1·9		1·8	0·9	1·7	1·8	3·5		0·8	0·8				1·5		0·8	1·8						0·2		
11	M	3·0	0·7	0·4	1·8		3·3		3·5			0·1		2·5		0·7		0·6		0·2			1·6				
12	H	1·7	0·4	0·6	4·0		1·9	1·3			0·6			0·9		0·1		0·6									
13	C	1·7	1·1	0·5	5·4		1·3		3·5		0·6			1·9	0·5	1·1	0·6	0·3		0·1	1·0		1·1	0·1	0·2		
14	M	1·0	1·0		1·5		3·6		1·7		0·4			3·0		0·5	0·5		0·5		0·2		0·7				
15	C		0·3	0·4	8·7						0·5			3·0		1·7	0·2	1·3		0·1		0·4	1·3	1·5	0·4		
16	H	4·6	1·2	0·9			9·4		0·4		0·4	0·4		0·6		1·5			1·5								
17	H	0·9	0·1	0·4	5·4		2·5	10·0			0·3	0·4		1·0		0·6			1·4								
18	H	2·8	0·5	0·7	4·3		4·0		3·0	4·0	1·0			0·7		0·4		1·5		0·1							

| 19 | Parsee | 26 | 13 | 01 | 45 | 76 | | | 07 | | 25 | | 17 | 04 | 30 | 30 | 04 | | 12 | | |

(*d*) vitamins A and C, and (*e*) in salts The amount of vitamin B present varies in the different groups Vitamin D is assumed to be obtained from exposure to sunlight

(5) The Hindu mill-workers have the most marked deficiency in fats and animal protein the grain eaten are unmilled and therefore the supply of vitamin B is good

(6) The diet of Group II (mothers of premature infants) is markedly deficient in vitamin B

(7) It is suggested that this relative deficiency in vitamins A and C or some factor associated with this deficiency, is concerned in the ætiology of 'pernicious anæmia of pregnancy,' and that the proportion of fat and vitamin B in the diet may play some part in determining the incidence of this disease in a population generally short of vitamins A and C

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STUDIES IN PERNICIOUS ANÆMIA OF PREGNANCY

Part III.

DETERMINATION OF NORMAL BLOOD STANDARDS FOR THE NUTRITIONAL LABORATORY'S STOCK ALBINO RAT

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Introduction

It is essential before undertaking any experimental work with animals to be certain that adequate normal standards are available for comparison with the experimental results Although there are blood standards for normal albino rats such as those published in Donaldson's (1924) book, it is necessary to establish the normal findings for each stock of rats, as the range of the normal in different stocks varies widely The present paper gives the standards for the nutritional research laboratories animals

Stock—The stock excluding all animals under experimentation is maintained at a daily average of approximately 700 animals, half male and half female of these about 100 of the female animals are pregnant During the last 12 months the death rate from natural causes among this stock has been nil Probably a few of the young die or are killed within the first week of life but as the litters are not disturbed it is impossible to estimate how many are lost, however as the average surviving litter numbers seven (maximum

litter born 15) the death rate cannot be high The growth curves of the young rats are normal on the usually accepted standards The stock therefore is considered healthy

Locality—Coonoor is situated at an altitude of 6,000 feet and has a moderate climate with no extremes of heat or cold The altitude should lead to a compensating increase in the average number of red cells in the blood

Housing and food—The animals are housed in airy but warm animal houses, and except when breeding are exposed regularly to sunlight Scrupulous attention to cleanliness is insisted on The stock diet consists of whole wheat, sprouted gram, carrots, cabbage, lettuce, diluted milk equivalent to approximately 5 c c of milk per rat per day, and butter Raw meat is added once a week and tomatoes are given occasionally The food is fed in excess Water *ad libitum* is provided in open pots, so that the animal can wash as well as drink

Breeding—The females are mated at maturity The male is removed at the birth of the litter and the young separated after 30 days

Methods—Peripheral blood was obtained from the tail it is essential to get free bleeding, for which purpose the tail was placed for several minutes in water at 40°C before snipping The same pipettes were used throughout the work

The red blood cells were diluted with Toisson's solution and counted in a Burker-Turk counting chamber Ten large squares were counted as a routine The white blood cells were diluted with 3 per cent acetic acid This stronger acetic solution gives more satisfactory results than the usual 0 5 per cent solution as the red cells are very resistant and do not dissolve readily in the latter It is essential to shake very vigorously to ensure proper distribution of the cells All the squares are counted, i e, 25 × 16 small squares The differential count was made in the counting chamber and checked by a count on the slide

The hæmoglobin estimation could not be done by the accurate colorimetric acid hæmatin method, as it was impossible to get large enough quantities of blood A modified Sahli was therefore used, the standard acid hæmatin solution being checked regularly against a known solution of hæmoglobin (Supplied by Major Sokhey and standardized by Van Slyke's method for oxygen capacity of blood) The error on the Sahli method even with corrected standard is known to be very high, and may be as great as 18 to 20 per cent the hæmoglobin values are therefore not as reliable as the blood counts

Preparation of slides—Three sets of slides were taken (1) unstained slides for estimation of cell diameter these should be thin, (2) slides made from blood drawn into the following solution saturated aqueous brilliant cresyl blue 5 c c 2 per cent sodium oxalate 1 c c, mixed and filtered These slides were used for the estimation of the percentage of reticulocytes, (3) slides stained with Giemsa for differential count and general examination

PLATE XXVI

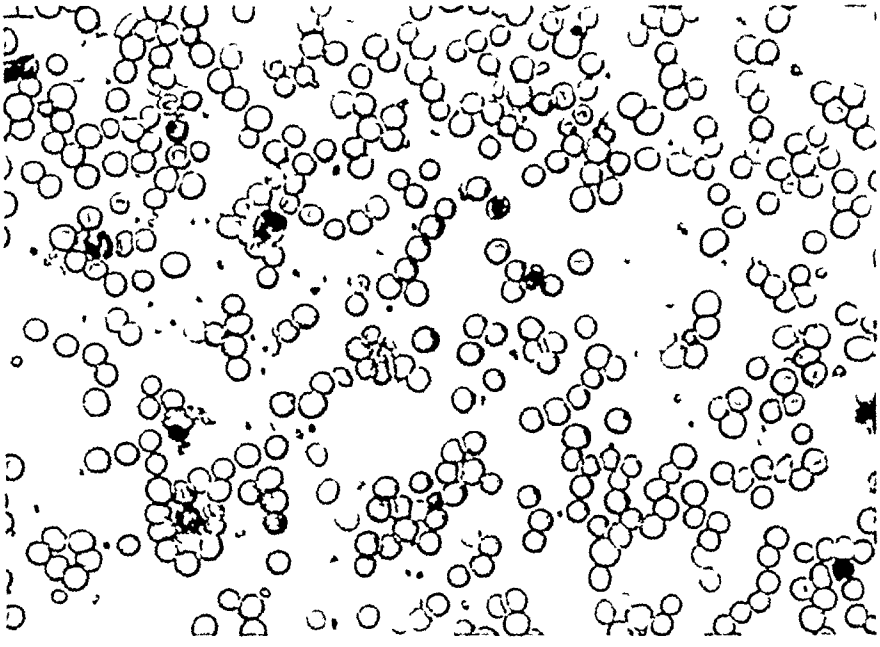


Fig 1—Blood film from 1 day old rat

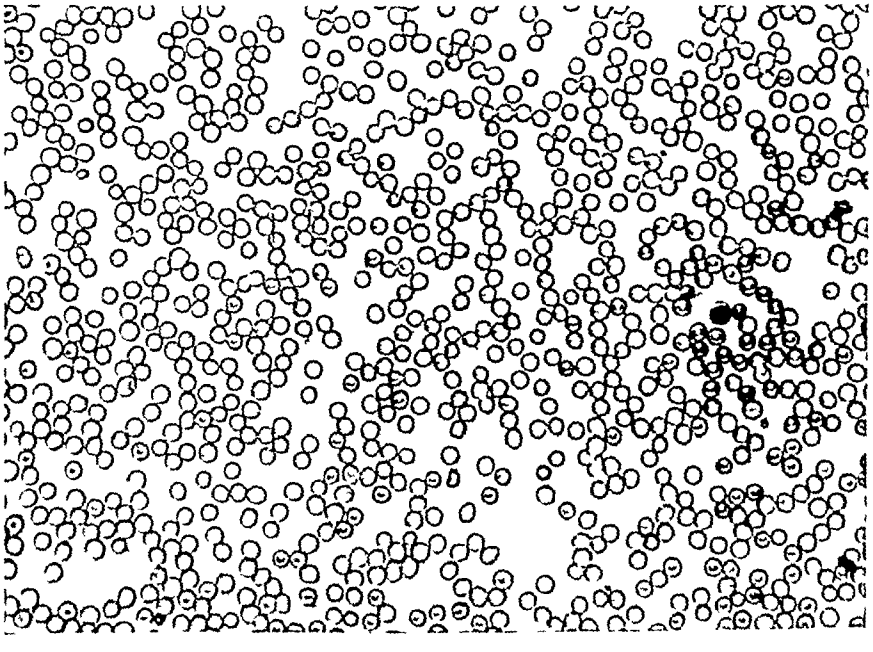


Fig 2—Blood film from mature rat

Method of measuring red cell diameter—A simple halometer method (Pryce, 1929) was used and standardized by using blood slides from which Price-Jones' curves had been made and the actual mean diameter of the cells determined

The instrument consisted of a modified Young's eriometer (Preston) as described by Pryce (1929) A source of light is placed behind a plate which has a small central aperture, 1—2 mm in diameter surrounded by a ring of 2 cm radius of pin point holes This is viewed through the blood film to be examined which is carried on a sliding rider which moves along a centimetre scale at right angles to the source of light A series of spectra of varying intensity are seen surrounding the central point of light and the circle of dots appear as a ring of luminous points The rider is moved till the yellow band of the brightest spectra coincides with the ring of luminous points The distance of the film from the plate at which coincidence occurs varies directly as the diameter of the particles on the film, i e, red blood cells Therefore, if the mean diameter of any set of corpuscles is known that of the unknown can be calculated by simple proportion The depth of the spectrum is a measure of the variability of the size of the particles and is therefore a measure of the degree of anisocytosis in any slide

Grouping of animals—Male and female (non-pregnant) animals were examined from birth to full maturity at 10-day intervals Animals aged between 5—14, 15—24 days, etc, were grouped together under the headings 10, 20, etc, respectively, but in the tables and charts the means of the exact ages are given, for example in age group '40' the mean of the ages is 39 days After 40 days males and females were separated but the differences in the mean value for the estimations done were not significant In addition a series of pregnant animals were studied

Results

I Males and Non-pregnant females

A *Red Blood Cells* Table I, Charts 1, 2 and 3 Plate XXVI

1 *Count*—At birth the count is 3 4 millions after an initial drop to 2 2 millions at 10 days, there is a steady rise to 9 55 millions at maturity (90 days) after which the values remain almost constant though there is a very slight increase with advancing age (up to 18 months) The rate of increase in the number of red cells is greater in the first 8 weeks of life, after which the cell curve flattens

2 *Mean diameter*—The average size of the red blood cells decreases steadily from birth to maturity As measured in terms of the mean diameter, the decrease is from 8 62μ to 5 86μ The range in size of the cells for an adult rat is shown in Chart 3

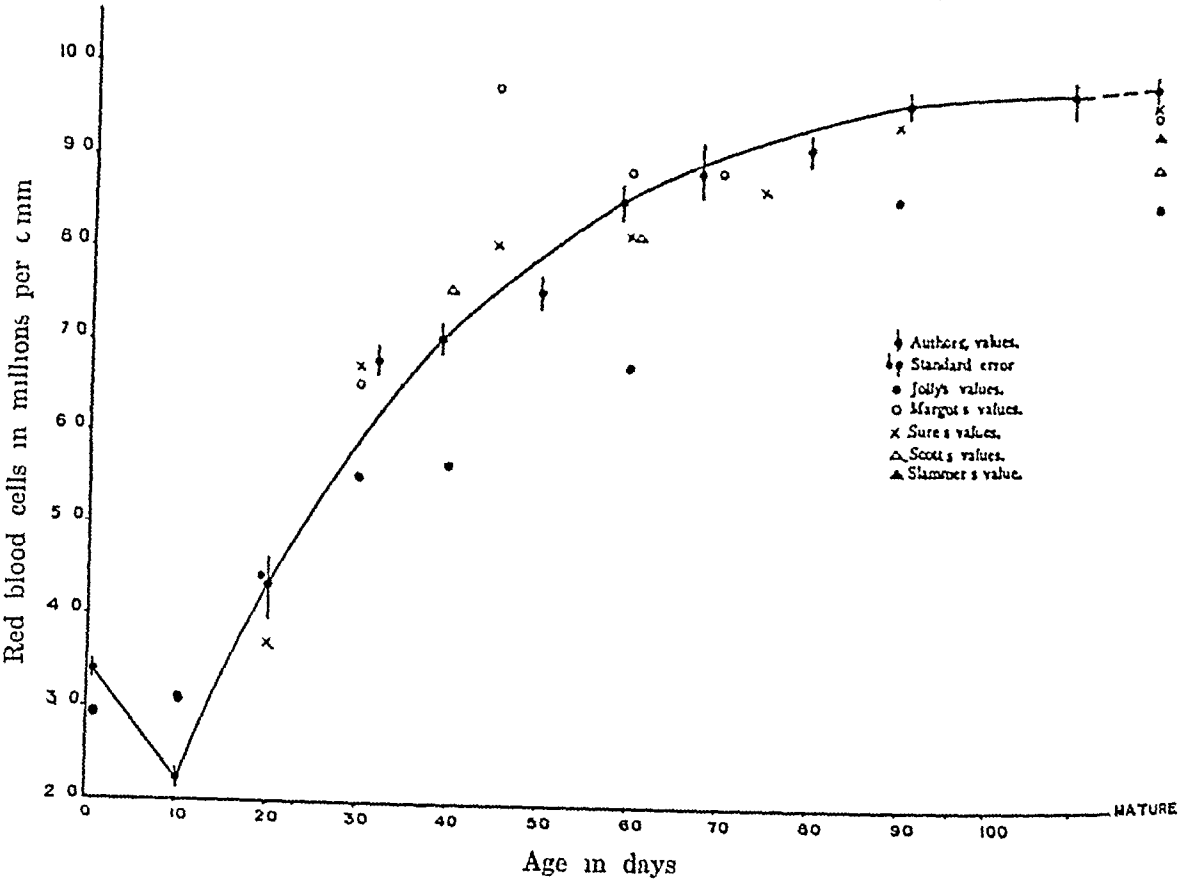
3 *Percentage of reticulocytes and presence of nucleated cells*—At birth there are a large number of immature cells in the circulation as shown by the presence of many reticulocytes (64 per cent of all reds), large numbers of normoblasts (104 per 100 white cells counted) and many polychromatophilic cells The nucleated cells disappear rapidly from the circulation but up till the 30th to 40th day there are appreciable numbers of reticulocytes, and a few polychromatic cells will be found even in slides from old rats

B *Hæmoglobin* Table I, Chart 2

The hæmoglobin content of the blood rises steadily after the initial drop during the first 10 days, from 11 01 grms at birth to 14 0 grms at maturity

CHART 1

Numbers of red blood cells on age Mean values



C *Colour Index* Table I, Chart 2

The standards for calculating colour index must be fixed with reference to the normal red cell count and hæmoglobin value for adult rats For this purpose we have taken 14 grammes hæmoglobin per 100 c c as 100 per cent hæmoglobin and for convenience 10 million red cells as normal red cell count In Table I the colour indices calculated on this basis are shown in one column and for comparison with values quoted in the literature we give in another column values based on the usual human standards (13 8 grms hæmoglobin and 5 0 million red cells) Owing to the large size of the red cells in young animals the colour index is raised at birth but has decreased to a practically constant figure of 1 00 (0 56 human standards) by the 60th day

D *White Blood Cells* Table I, Chart 4

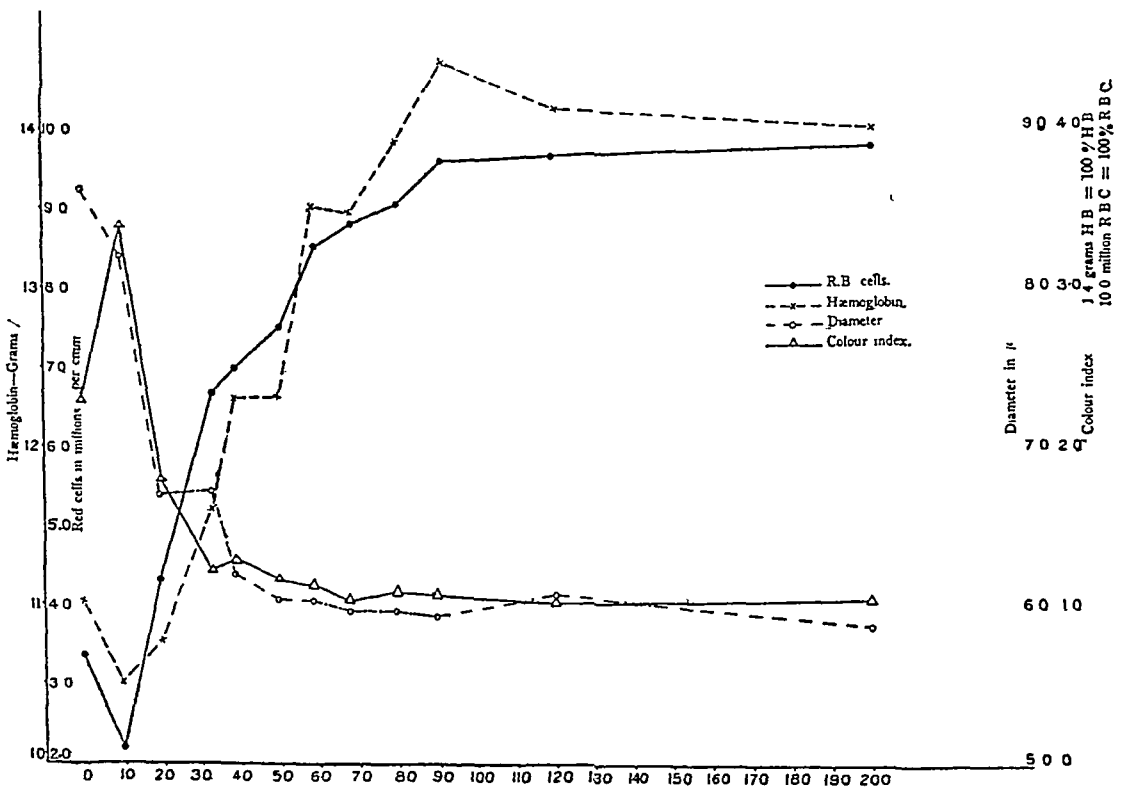
1 *Count*—The variation in the white cell count is very great, not only age groups but in individuals For this reason it is very

difficult to attach any significance to variations in the counts unless the differences are very great As a whole after the initial drop in the count there is a steady increase in the number of white cells from birth to maturity

2 *Differential count*—In the Coonoor rats the differential count shows a marked preponderance of lymphocytes over the polymorphonuclear elements—the latter increasing slightly however with advancing age There are generally

CHART 2

Red blood cells, hæmoglobin, diameter of red blood cells and colour index on age
Mean values



speaking between 60 to 70 per cent small lymphocytes and 8 to 10 per cent large lymphocytes and transitional cells The eosinophiles (about 0 5 per cent) are characterized by their ring-shaped rather than lobulated nucleus

E *Platelets*

No observations were recorded as it is extremely difficult to make accurate counts of these elements in rats' blood

Pregnant animals Table I

Females of different ages and in different stages of pregnancy, some pregnant for the first time and some in their third or fourth pregnancy are

Table I

Blood findings in 220 Normal Albino Rats.

Age groups in days	Mean age in days	Number of observations	Sex	Red blood cells: Mean value	Standard deviation of mean	Minimum value	Maximum value	Diameter in μ: Mean	Diameter in μ: Standard deviation of mean	Reticulocytes % of total count	Normoblasts per 100 W B C counted	Anisocytosis	Polychromatophilia	Hæmoglobin: Mean in grammes %	Hæmoglobin: Standard deviation of mean	Colour index: I	Colour index: II	White blood cells: Mean	White blood cells: Standard deviation of mean	White blood cells: Minimum value	White blood cells: Maximum value	Differential count %: Polymorphonuclears Neutrophils	Differential count %: Polymorphonuclears Eosinophiles	Differential count %: Small lymphocytes	Differential count %: Large lymphocytes
				In millions per c mm														Per c mm							
1	1	4		3·42		3·15	3·83	8·62 (4)		64 (4)	104	++	++	11·04		2·30	1·20	7,350		6,300	8,400	36·0		53	11
10	10	4		2·21		2·09	2·37	8·2 (2)		15 (4)	2	++	++	10·49		3·39	1·60	1,600		4,200	5,100	14·0	·	70	16
20	20	6		4·33	0·870	2·88	5·22	6·7 (2)		10 (5)	0	+	±	10·76	0·810	1·78	0·93	3,500	825	2,600	5,000	16·0	1·0	65	18
30	32	26		6·74	0·755	4·67	8·30	6·73 (10)	0·230	7·3 (14)	0	±	±	11·59	0·622	1·23	0·61	6,785	2,130	3,600	12,000	17·0	·	73	10
40	39	39		7·00	0·854	5·20	8·84	6·20 (9)	0·198	4·2 (8)	0	±	±	12·28	0·975	1·26	0·66	8,320	1,900	5,000	12,000	21·5	0·5	69	9
50	50	28		7·48	0·940	5·62	9·35	6·04 (5)	0·185	1·6 (9)	0	±	−	12·28	1·018	1·18	0·61	7,500	2,140	4,800	12,400	20·5	0·5	71	8
		10	M	7·52	0·884	5·62	8·66							12·40	0·779								..	.	
		18	F	7·43	0·798	6·28	9·35							12·10	1·090				.		..	..	.		.

60	59	22		8·49	0·798	7·07	10·55			3·0 (7)	0	±	—	13·48	0·848	1·14	0·57	8,879	2,570	5,600	14,600	22·0		69	9
		12	M	8·64	0·745	7·23	9·55	6·12 (8)	0·240					13·58	1·450										
		10	F	8·39	1·076	7·07	10·55							12·80	1·470										
70	68	15		8·79	0·874	8·13	11·12	5·97 (14)	0·170	2·0 (9)	0	±	—	13·45	1·017	1·05	0·56	10,960	3,700	7,200	17,400	21·0	·	69	9
80	80	12		9·056	0·618	8·13	10·08	5·97 (22)	0·200	5·0 (7)	0	—	—	13·93	0·872	1·10	0·56	12,366	4,406	6,200	20,400	22·0	1·0	70	7
90	91	20		9·55	0·570	8·33	10·56	5·92 (19)	0·196	2·4 (10)	0	—	—	14·42	0·729	1·08	0·56	11,350	4,010	6,200	22,200	24·0		68	8
		10	M	9·58	0·466	8·80	10·50							14·21	0·648										
		10	F	9·53	0·657	8·33	10·56							14·62	0·764										
120	120	10		9·62	0·598	8·95	10·55	6·07 (5)	0·103	2·3 (5)	0	—	—	14·10	0·542	1·04	0·53	11,980	3,655	6,800	17,200	27·5	0·5	65	7
6 months and over		14		9·71	0·614	8·40	10·90	5·86 (11)	0·098	0·8 (4)	0	—	—	13·99	0·679	1·04	0·53	11,930	3,860	6,200	20,000	28·5	1·5	62	8
Adult females		17	F	9·43	0·648	8·30	10·60	5·91 (9)	0·096	0·7 (2)	0	—	—	14·19	0·910	1·07	0·54	10,980	3,454	6,000	17,200	26·0	·	67	7
Pregnant females		20	F	9·03	0·853	7·17	10·60	5·77 (16)	0·085	0·8 (5)	0	—	—	13·50	1·090	1·06	0·54	10,880	3,222	6,000	15,600	38·0		53	9

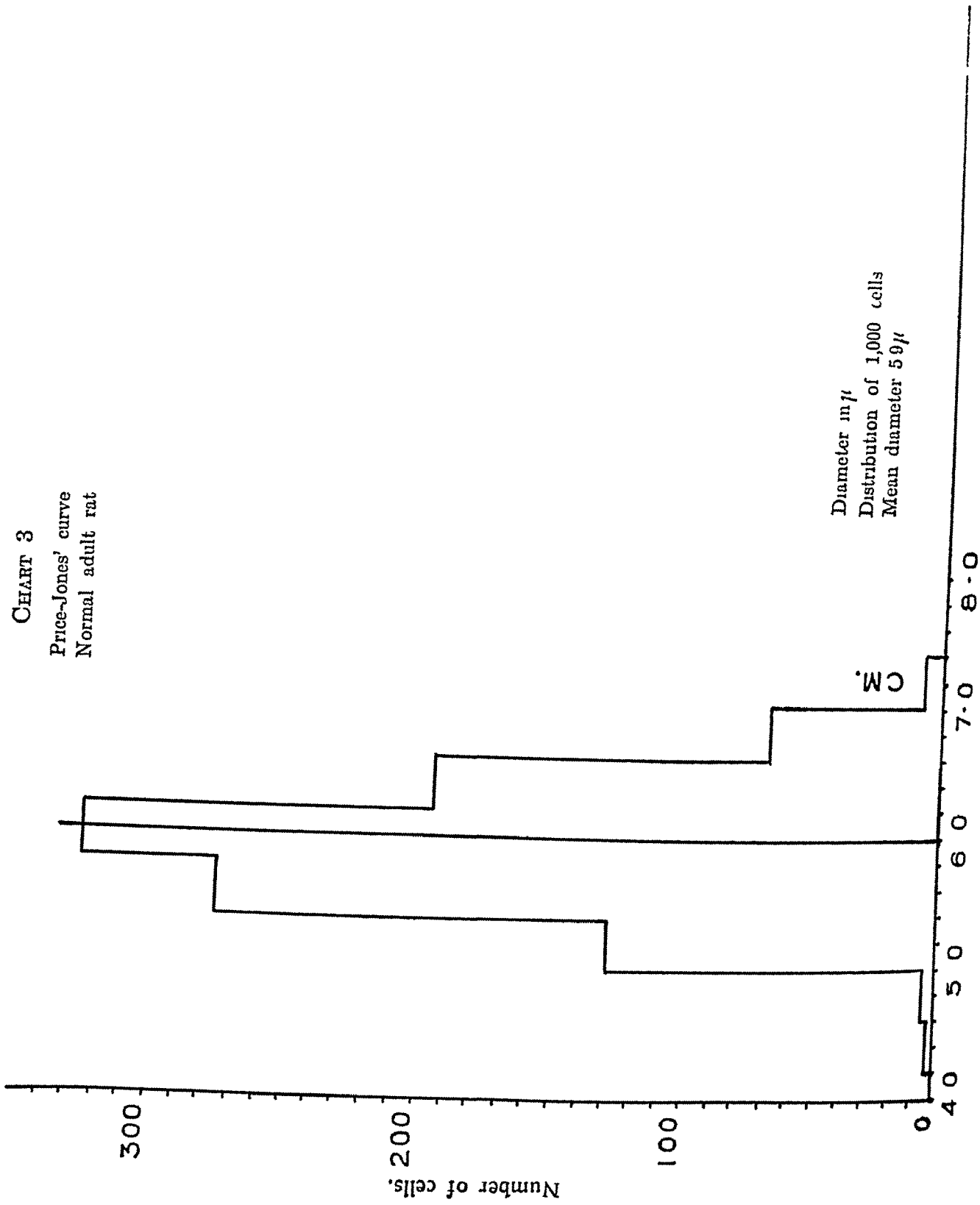


CHART 3
Price-Jones' curve
Normal adult rat
Diameter in μ
Distribution of 1,000 cells
Mean diameter 5·9μ
C.M.
Number of cells.
300
200
100
0
4·0
5·0
6·0
7·0
8·0

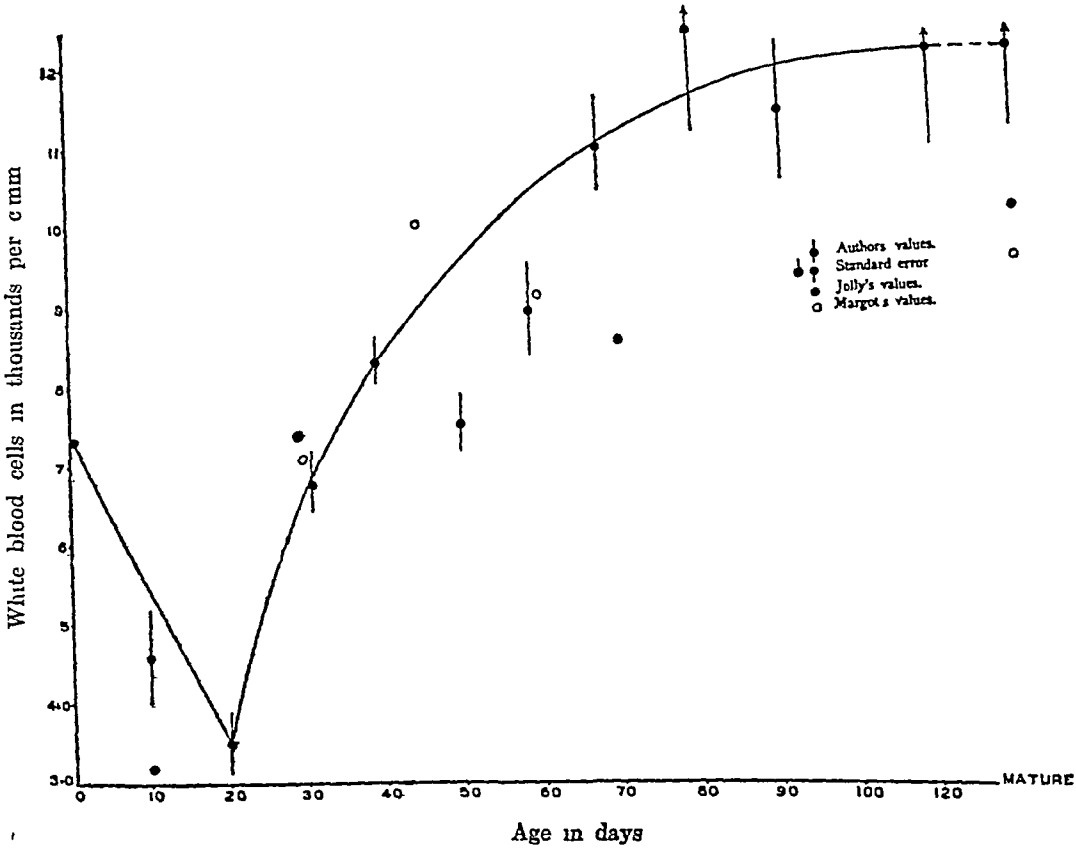
included in this group The majority of the counts were however taken within 24 hours of delivery, when these animals should show any changes in the blood findings that may occur owing to the strain of pregnancy Seventeen females of the same age group (90 days to maturity) were examined as a control series It is obvious from the figures that there is no significant difference between these two groups and that with the exception of a very slight lowering of the average count and hæmoglobin values, the animals are unaffected by pregnancy

Discussion

The findings set out above are quite distinctive and with the exception of the white cell counts very constant for each group considered Steady changes are demonstrated from birth to maturity The red cell count and hæmoglobin

CHART 4

Number of white blood cells on age Mean values



percentage after the initial drop rise steadily, and this increase is associated with a decrease in the average size of the red cells so that the colour index falls at first to become more or less constant at about 1 00 by the 70th day Too few animals were examined to be certain that the initial drop in the red

and white counts and hæmoglobin percentage is a constant finding but on the analogy of the human subject it is possibly so

The female animals have slightly lower red cell counts and hæmoglobin values but the differences are not significant and the pregnant females have a slightly lower mean red cell count and hæmoglobin percentage but compared with a similar group of non-pregnant animals there is no significant difference between the groups

A comparison of our results with those quoted in the literature (4—10) indicates that as far as red cell counts, hæmoglobin values and the white cell count are concerned, that there are no very significant differences between our findings and those of other authorities (*see* Charts 1 and 2)

As remarked by Donaldson (1924) the differential counts vary markedly from stock to stock—the American figures in general showing a higher percentage of polymorphonuclear cells than the English Our values for these polymorphonuclear elements are even lower than those quoted by English authors but as they were obtained not only by counts on the slide but also by counts made in the counting chamber and were absolutely consistent, we are of the opinion that this is a true finding for our stock

There are only a few figures for the diameter of the red cells given in Donaldson's book and the authors quoted, Treadwell, Wormley, Gulliver and Jolly all give figures for the mature rat that range between 6 5 to 7 0μ. Our figure is lower than this, approximately 6 0μ, but as it was obtained both by the direct measurement method and the halometer method, it probably is the correct figure for our stock

The question of the effect of pregnancy on the blood is of considerable interest, especially with reference to the effect of deficient feeding on pregnant animals The stock females in our series show no significant difference from similar non-pregnant animals There is a lowering of the red cell and hæmoglobin count at the end of pregnancy, but this is very slight The red cell count in one of the 20 animals examined falls below 8 0 million but values as high as 10 80 occur and the mean figure is 9 0 million The control group has an average red cell count of 9 4 million and excluding the one low value in the pregnant group the range is the same in the two groups The difference in the hæmoglobin values is slightly greater but as has been remarked before the error of the method is much greater These results are directly opposed to those of Sure and co-workers (1929) who report a severe anæmia with figures as low as 4 8 million for the red cell count in their pregnant animals It is difficult to explain these discrepant findings but it is strange that animals should become so anæmic during a normal physiological process such as pregnancy, if their hygienic conditions and diet are really adequate Further work on this question would be interesting

In conclusion we wish to thank Colonel McCarrison for permission to work in the Nutritional Laboratory and to use the stock animals

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OBSERVATIONS ON THE CORRELATION BETWEEN THE INCIDENCE OF FILARIAL INFECTION IN THE HUMAN HOST AND IN THE INSECT CARRIER IN RELATION TO TERRAIN

Part V

BY

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IV DISCUSSION OF RESULTS
V CONCLUSIONS

I INTRODUCTION

THE investigation into filariasis was undertaken under the auspices of the Indian Research Fund Association, Simla, the areas in Bihar and Orissa were mapped out for observation and Gaya was made the centre of the Inquiry

The object of the investigation has been to study the existence of any correlation there may be between the incidence of human infection, and infection in the invertebrate host in relation to the physiography and physical characters of an area

The first point has been to determine the most probable invertebrate host of *F bancrofti* under the conditions prevailing in this particular locality.

The invertebrate host of *F bancrofti* seems to differ in different countries Workers in this field of research have found that, although *F bancrofti* develops to a certain extent in different species of Culicine mosquito, only one or two species act as definite intermediary or carrier hosts *par excellence* Low, in 1901, carried out feeding experiments on human cases harbouring *F bancrofti* in St Lucia with *Aedes argenteus* (Poir) He found that only partial development took place in the thoracic muscles and that no worms reached the infective stage or migrated into the proboscis of the mosquito

Three other species of *Aedes* (*Stegomyia*) are given by Hindle (1914) as being probable carriers of *F bancrofti* in Malaya, but he states that forms have not reached the infective stage or been seen in the proboscis (Filarial Commission, 1921).

Manson-Bahr has observed that developmental forms of Fijian microfilaria (morphologically identical with microfilaria *bancrofti*, but observes no periodicity) are found in *Culex fatigans, Stegomyia pseudoscutellaris, Stegomyia fasciata,* and *Culex jepsoni* in Fiji

While capable of developing in *Culex fatigans,* the favourite intermediary of *F bancrofti,* this mosquito is not nearly so efficient an intermediary in Fiji as it is in other countries or as is *Stegomyia pseudoscutellaris,* the common mosquito of the group of islands

The results of the Filariasis Commission in British Guiana under the direction of Prof Leiper show that *Aedes argenteus* (Poir) (*Stegomyia fasciata*) is an unsuitable intermediary host for *Filaria bancrofti* In the opinion of the Commission the invertebrate host is the domestic mosquito *Culex quinquefasciatus* Say (*Culex fatigans* Wied)

Almost in all cases the assumption that a particular species of mosquito acts as efficient intermediary is based on the finding of the infective developmental stage of *bancrofti* in that intermediary (I am not aware of any direct transmission experiment)

Coming now to certain areas in British India, it has been observed that the conditions for development of *Filaria bancrofti* are fulfilled in *Culex fatigans,* the prevalent species of domestic mosquito in many of the areas of Bihar and Orissa (Korke, 1928 and 1929)

In order to determine the actual vector of *bancrofti,* two methods are possible The first method is direct and must be based on actual transmission of the parasite to the definitive host through its intermediary The second method is indirect and based on the epidemiological evidence in the incidence of filariasis and on the distribution of the vector in which the conditions of

development of the infective forms of *bancrofti* are fulfilled The present investigation in this direction has been approached through the latter method

It has previously been observed that informative results are likely to be obtained by studying the conditions of incidence of *F bancrofti* in different groups of the population in relation to the physiography and physical characters of the country (Korke, 1928 and 1929) As to physical characters, the type of the cultivation appears to be a reliable test It indicates the agricultural resources of a soil, which may vary in different localities, the variation depending especially upon the degree of retention of moisture

Basing the investigation on these grounds, the province of Bihar and Orissa has hypothetically been divided into 'Belts' according to the physical and physiographical conditions

II Material and technique

The blood material for this paper was chiefly derived from the agricultural section of the population The technique of making blood films, staining, collection and dissection of mosquitoes has already been described (Korke, 1928)

The species of mosquitoes were identified for me by Captain P J Barraud, of the Malaria Survey of India, Kasauli, to whom my grateful thanks are due

I also acknowledge the assistance given to me by my assistants Dr K K Das and S A S Babu Kulamoni Misra

My grateful thanks are also due to many of the officials and non-officials in Bihar and Orissa and especially to the Police officials who so willingly helped the Inquiry

III Results obtained in field studies

(*a*) *Bihar and Orissa divided into belts*

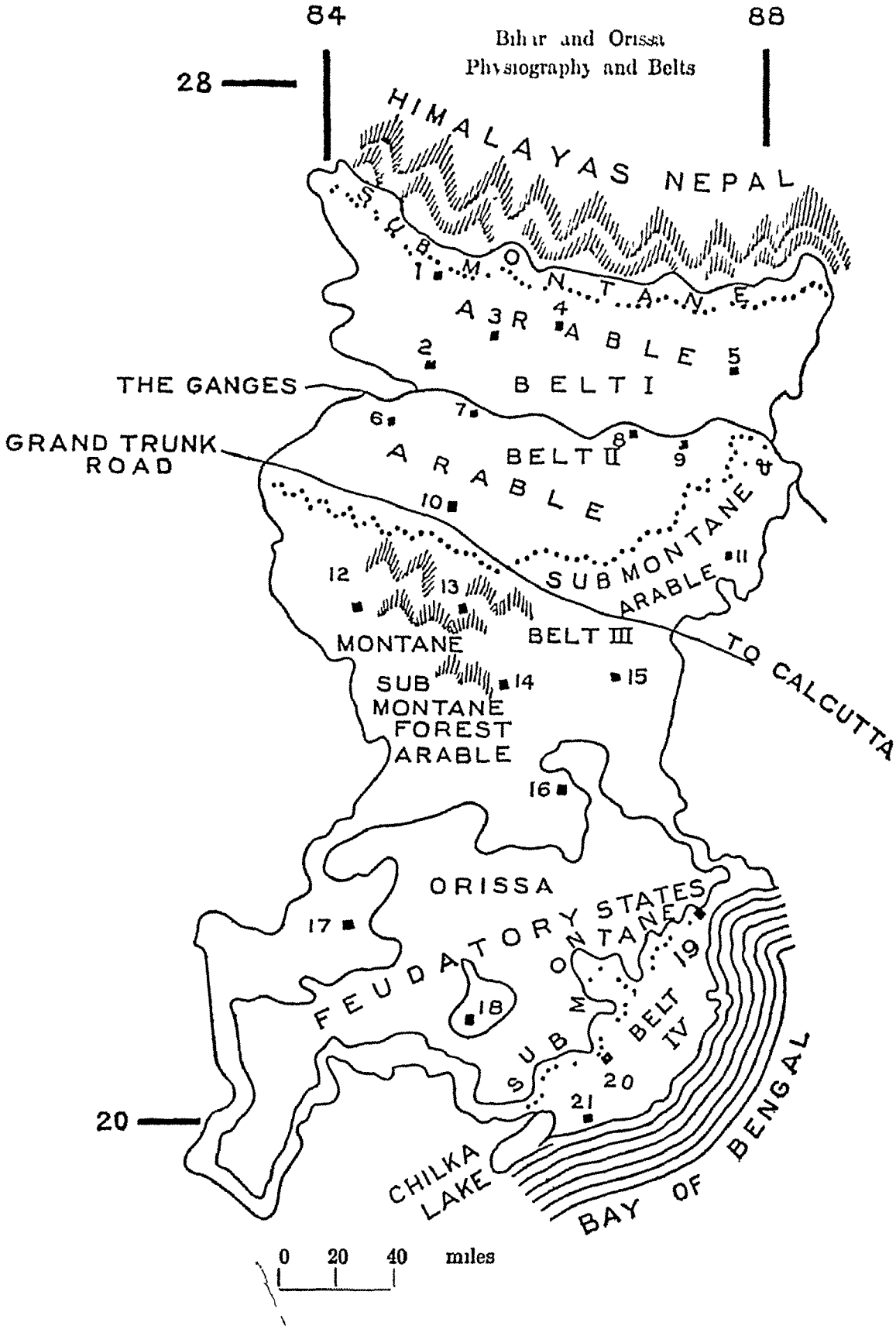
The province is almost a parallelogram in shape, situated between 84°—88° East Longitude and 28°—20° North Latitude The south-east corner of the province is pinched off by the Bay of Bengal For the purpose of investigation it may be divided into three transverse parallel belts and one oblique belt on the sea coast, which forms the south-east corner of the area (*see* Map)

The northern belt (Belt I) encloses an area between the Nepal Himalayas and the Ganges The northern fringe of this belt is a sub-montane tract and the rest of the belt is rich arable land This area is intersected by large tributaries of the Ganges The chief cultivation is paddy

The middle belt (Belt II) encloses an area between the Ganges to the north and a fringe of the Chota-Nagpur plateau to the south Except for some portion of the sub-montane tract in the Santal Parganas, the area is a rich arable land the chief cultivation being paddy

Both these belts go to form the Gangetic plain, and the plain is slightly above the areas at sea-level

MAP



Belt I

District Areas

1 Champaran
2 Saran
3 Muzaffarpur
4 Darbhanga
5 Purnea

Belt II

6 Shahabad
7 Patna
8 Monghyr
9 Bhagalpur
10 Gaya
11 Santal Pargana

Belt III

12 Palamau
13 Hazaribagh
14 Ranchi
15 Manbhum
16 Singhbhum
17 Sambalpur
18 Angul

Belt IV

19 Balasore
20 Cuttack
21 Puri

The southern belt (Belt III) forms the bulk of the province and consists of the montane, sub-montane and forest areas The belt encloses an area of the Orissa Feudatory States The land is arable in the alluvial tracts which are extensive and paddy forms the chief cultivation

The sea coast belt (Belt IV) is limited by the sea towards the east and sub-montane tracts of land towards the west The country is well watered by rainfall, rivers and canals and the arable land is rich in paddy cultivation

The temperature and rainfall of these belts vary according to seasons In some parts the climate is warm, in some temperate, in others moderately cool

The agriculture in all the belts depends upon the rainfall, canal and river inundations

Prevalent species of mosquitoes

The mosquitoes were caught, as they were found, inside and outside a dwelling and no special effort was made to collect only a particular species If therefore there were more dissections done on Culicines or on Anophelines respectively in a belt, the fact was due to the prevalence in numbers Only female mosquitoes were dissected Wherever the developmental forms of *bancrofti* were found this was invariably in *Culex fatigans* The total number of Anophelines and Culicines dissected in each belt is shown under Table I The following prevalent species of mosquitoes were identified in the belts of Bihar and Orissa and the number of belt is given against each species *Aedes* (*Stegomyia*) *albopictus* (II), *A* (*Stegomyia*) *aegypti* (II), *A* (*Stegomyia*) *vittatus* (IV, III, II), *A* (*Skusea*) *microperus* (IV), *A* (*Aedimorphus*) *piperisalatus* (II, III), *A* (*Aedimorphus*) *pallidostriatus* (III), *A* (*Banksinella*) *lineatopennis* (III), *A* (*Finlaya*) *gubernatoris* (III), *Armigeres obturbans* (IV), *Culex fatigans* (I, II, III, IV), *C* (*Culiciomyia*) *pallidothorax* (II), *C* (*Culiciomyia*) *pullus* (II), *C bitaeniorhynchus* (II, III, IV), *C vishnui* (II, III, IV), *C whitmorei* (II, III, IV), *C epidesmus* (II, III, IV), *C gelidus* (II, III) *C sitiens* (II), *C tritaeniorhynchus* (II, III), *Lutzia fuscana* (II, IV), *Lutzia raptor* (III), *Mucidus scataphagoides* (III), *Taeniorhynchus* (*Mansoniodes*) *uniformis* (II, III, IV), *T* (*Mansoniodes*) *annulifer us* (IV), *Anopheles fuliginosus* (I, II, III), *A pallidus* (II, III), *A subpictus*(II, III), *A culicifacies* (II, III), *A listoni* (III), *A hyrcanus* (II, III), *A maculipalpis* (III), *A jeyporiensis* (III), *A stephensi* (II), *A vagus* (III)

(b) *Filarial incidence in relation to the physiography and insect carrier, arable area, Gangetic plain, Belt II*

The incidence of infection in Belt I is given under Section IV, as no mosquitoes in correlation with the human incidence were dissected from this belt

Belt II, representative type area, Gaya district, the area is about 5,000 square miles and forms the southern portion of the Patna division The

physical characters of this area have already been described (Korke, 1929, *Transaction, F E A T M*)

The sections of the population investigated in this belt were, General, Police, School and Agricultural The incidence of human infection was due to *F bancrofti*

Areas investigated

Area 1, urban, Gaya town, regular police force, total cases, 102, positive, 13, 13 per cent (16-6-28) Incidence of infection in this area was found to be 16 per cent, (Korke, 1926, 1927, 1928)

Two areas for observation were selected from the old Gaya town Total mosquitoes dissected, 334, positive, 46, 14 per cent (November to August 1928)

A second observation was conducted in the identical area Total *C fatigans* dissected, 23, positive, 7, 30 per cent, (16-5-29)

A third observation was made in the same area Total *fatigans* dissected, 34, positive, 5, 15 per cent, (6-6-29)

Area 2, suburban, Nabinagar, total cases, 72, positive, 7, 10 per cent, (23-3-29) Total *fatigans* dissected, 68, positive, nil

Area 3, suburban, Tekari, total cases, 165, positive, 27, 16 per cent, (26-9-28) Total *fatigans* dissected, 22, positive, 1, 4 5 per cent Anophelines, 2, positive, nil, (19-5-29) Second observation, total *fatigans* dissected, 84, positive, 16, 19 per cent, (27-7-29)

Area 4, suburban, Nawadah, total cases, 57, positive, 6, 10 per cent, (5-10-28) Total *fatigans* dissected, 12, positive, 10, 24 per cent, (11-7-29)

Area 5, suburban, Sherghatti, total cases, 61, positive, 8, 13 per cent, (18-6-28) Total *fatigans* dissected, 35, positive, nil, Anophelines, 13, positive, nil, (30-8-28) Another observation (12-6-29) Total *fatigans* dissected 54, positive, 7, 13 per cent, Anophelines, 2, positive, nil

Area 6, suburban, Daudnagar, on the Sone Canal Total cases, 118, positive, 12, 10 per cent, (24-11-27) Total *fatigans* dissected, 94, positive, 11, 12 per cent, (30-5-29)

Area 7, suburban, Aurangabad, total cases, 279, positive, 14, 5 per cent, (7-11-27, 13-9-28 and 7-12-28) Total *fatigans* dissected, 32, positive, 1, 3 per cent, (19-3-29)

Area 8, village, Jamhor, a prosperous village of some importance Total cases, 71 (backward and poor class of agriculturist), positive, 20, 28 per cent, (16-9-28), time, night Total cases, 94 (school boys and better class), positive, 4, 4 per cent, (10-12-28), time, night Total cases, 50 (same as above), positive, nil, time, day Total *fatigans* dissected, 252, positive, 18, 7 per cent, total Anophelines dissected, 173, positive, nil, (11-12-28 and 25-5-29)

Area 9, village, Piperdih (Aurangabad), inhabited mostly by the cowherds Total cases, 44, positive, nil, (15-3-29) Culicines dissected, 28, positive, nil, Anophelines dissected, 11, positive, nil, (16-3-29)

Area 10, village, Khakra (Aurangabad), total cases, 60, positive, 1, about 2 per cent, (19-3-29) Culicines dissected, 58, positive, nil, Anophelines dissected, 4, positive, nil

Area 11, village, Goh, total cases, 103, positive, 8, 8 per cent, (7-5-29) Culicines dissected, 25, positive, nil, Anophelines dissected, 1, positive, nil, (11-5-29)

Area 12, village, Wazirganj, total cases, 102, positive, 20, 20 per cent, (19-10-27) Total *fatigans* dissected, 46, positive, nil, Anophelines, 30, positive, nil, (31-9-28) Second observation, (27-7-29) Total *fatigans* dissected, 50, positive, 7, 14 per cent

Area 13, village, Gurwak, total cases, 52, positive, 3, 6 per cent, (9-1-28) Total *fatigans* dissected, 41, positive, 2, 5 per cent, (10-7-29)

Area 14, village, Barachatti, total cases, 60, positive, 6, 10 per cent, (9-10-27) Total *fatigans* dissected, 5, positive, 1, (13-6-29)

Area 15, suburban, Bihar, total cases, 60, positive, 9, 15 per cent, (14-1-28) Total *fatigans* dissected, 80, positive, 26, 33 per cent, (16-7-29)

(c) *Filarial incidence in relation to the physiography and insect carrier, arable area, montane and sub-montane tracts, Belt III*

Representative area, Hazaribagh district and part of the Ranchi plateau The physical characters of the area have already been described (Koike, 1929, *Transactions, F E A T M*)

The absence of water is the most striking feature in the scenery of the lower plateau, but on the higher plateau the country is open and the cultivation fairly extensive The surface is never level, there are no lakes and marshes in the district

The Damodar basin divides Hazaribagh from the Ranchi plateau, the physical characters of which resemble mostly those of Hazaribagh The incidence of human infection in this belt was due to *F bancrofti*

Area 1, urban, Hazaribagh town, situated on the higher plateau about 2,000 feet above the sea-level The place is supposed to be a sanatorium

Population, police recruits, total cases, 69, positive, 13, 19 per cent, (24-6-29) Total *C fatigans* dissected, 46, positive, 8, 17 per cent Two subpictus, positive, nil

Area 2, urban, Ranchi town, (Ranchi plateau about 2,000 feet above the sea-level) Total cases, 111, positive, nil, (16-1-29)

Area 3, village, Barhi, (lower Hazaribagh plateau on the Grand Trunk Road) Total cases, 54, positive, nil, (19-5-28 and 21-12-28) Total Anophelines dissected, 13, positive, nil, second observation (25-6-29) Total *fatigans* dissected, 8, positive, 2

Area 4, village, Barakattha, (lower plateau, Grand Trunk Road) Total cases, 50, positive, nil, (20-5-28)

Area 5, village, Bagodar, (lower plateau, Grand Trunk Road) Total cases, 79, positive, nil, (24-12-28) Total *fatigans* dissected, 7, positive, nil,

Anophelines dissected, 225, positive, nil, (21-12-28) Second observation (23-6-29) Total Culicine dissected, 69 (60 *C fatigans*), positive, 1, Anophelines dissected 11, positive, nil

Area 6, village, Dhumri, (lower plateau, Grand Trunk Road) near the foot of the Parasnath Hill Total cases, 38, positive, nil, (17-9-28) Total Culicines dissected, 7, positive, nil

Area 7, village, Topchanchi, (Grand Trunk Road) near the Parasnath Hill Total cases, 105, positive, 3, incidence, 3 per cent, (30-12-28) Total Culicines dissected, 2, positive, nil, Anophelines dissected, 86, positive, nil

Area 8, industrial, Kodarma, (lower plateau) Total cases, 134, positive, nil, (12-10-28) Total Culicines dissected, 10, positive, nil, (13-9-28) Total *C fatigans* dissected, 55, positive, 4, 7 per cent, (27-7-29)

Area 9, village, Muzagunge, total case, 1, positive, nil, (16-9-28) Total Culicines dissected, 7, positive, nil

Area 10, village, Mandu, (higher plateau) Total cases, 156, positive, nil, (12-1-29) Total Culicines dissected, 1, positive, nil, Anophelines dissected, 116, positive, nil, (12-1-29) Total Culicines dissected, 8, positive, nil, (30-9-29)

Area 11, village, Ramgarh, (Damodar basin) Total cases, 48, positive, nil, (9-1-29) Total Culicines dissected, 8, positive, 2, Anophelines dissected, 30, positive, nil, (26-1-29)

Area 12, village, Gola, (Damodar basin) Total cases, 60, positive, nil, (9-1-29) Total Anophelines dissected, 15 positive, nil, (10-1-29)

Area 13, suburban, Chatra, (lower Hazaribagh plateau) Total cases, 96, positive, 2, incidence, 2 per cent, (7-10-28) Mosquitoes were identified but not dissected

Area 14, village, Ormanjee, (Ranchi plateau) Total cases, 60, positive, nil, (14-1-29) Total mosquitoes dissected (species not determined), 22, positive, nil

Area 15, village, Khunti, (Ranchi plateau) Total cases, 108, positive, nil, (21-1-29) Total mosquitoes dissected (species not determined), 24, positive, 1, (21-1-29)

Area 16, village, Chauparan, (lower Hazaribagh plateau, Grand Trunk Road), about 800 feet above the Gaya plain Total cases, 12, positive, 1, (19-10-28) Total *fatigans* dissected, 34, positive, 5, 15 per cent, (27-7-29)

(*d*) *Filarial incidence in relation to the physiography and insect carrier, arable area, sea coast, Belt IV*

The Littoral area comprises the three districts of Orissa, Balasore, Cuttack and Puri

Representative area, Balasore district

The observations on (1) physiography and physical characters of this area, (2) the incidence of filarial infection in the sections of population of the

urban, suburban and village areas, (3) the prevalent species of mosquitoes and the study of developmental forms of *bancrofti* have already been published (Korke, 1929, Part III)

(*c*) *Observations on the invertebrate host*

Culicine mosquito the intermediary

Out of the 1,504 Anophelme mosquitoes (1,207 identified), 874 females showed uniformly negative results Out of the 3,739 Culicine mosquitoes (2,672 identified), 2,132 showed positive results in 221 or 10 per cent

The observations showed that if a mosquito is the only carrier, then a Culicine mosquito is the vector

Culex fatigans the vector

The analysis of the results shows that 101 mosquitoes dissected at Puri, but not identified, showed positive results in 12 (Korke, 1928, Part II)

Three hundred and forty-nine mosquitoes dissected in the Balasore area, but not identified at the time of dissection, showed positive results in 29 The specimens kept for identification were *C fatigans*, *C vishnui*, *T* (*Mansonioides*) *annulifer us* and *uniformis* and *A* (*Skusea*) *micropter us* (Korke, 1929, Part III)

Two hundred and fifty-eight mosquitoes dissected in Gaya showed positive results in 39 All specimens kept for identification were *C fatigans* (Korke, 1928, Part II)

One thousand four hundred and twenty-four mosquitoes identified at the time of dissection showed positive results in 141 *C fatigans*

C fatigans may safely then be accepted as the intermediate host of *F bancrofti* in the areas of Bihar and Orissa

Developmental stages of *bancrofti* found

The developmental stages of *bancrofti* as found in the mosquito have already been described (Korke, 1928, Part II)

A morphological study of the developmental forms in 148 *C fatigans* demonstrated that 41 mosquitoes or 27 7 per cent showed the first or the human phase, where embryos have cast off their sheath in the stomach but retain the original characters as are found in the human peripheral blood, 56 or 37 7 per cent the phase of sausage-shaped bodies and 31 or 20 9 per cent the phase of cylindrical bodies, phases where embryos undergo developmental changes in the thoracic organs, 20 or 13 5 per cent the filariform or infective phase, where they elongate over lengths of one millimetre, possess alimentary tract and active movements and 3 mosquitoes showed the proboscis stage

C fatigans collected from the interior of houses

It has been mentioned that in the earlier stages of survey, mosquitoes were mostly collected from outside the premises To observe the exact condition

of infection, areas in a few instances were revisited and mosquitoes were collected from the interior of houses

In Belt II, mosquitoes when collected from outside the premises showed species of Anophelines and Culicines in a mixed number but when caught from inside the houses they were mostly *C fatigans* *C fatigans* showed the infection ranging between 0 to 7 per cent, when caught from outside the houses (8 areas) and 7 to 33 per cent when caught from inside the houses (8 areas)

In Belt III, mosquitoes when collected from outside showed prevalence of Anophelines over Culicines In fact Culicines and *C fatigans* were collected in very small numbers and showed negative results Four areas were revisited, viz, Barhi, Bagodai, Kodarma and Chauparan, *C fatigans* collected from inside the houses, showed infection in 2 out of 8, 1 out of 76, 5 out of 34 and 4 out of 55 in the above areas respectively

The observation is interesting from the standpoint of collecting the right type of material from the interior of houses for investigation purposes

Seasonal prevalence

Culex fatigans has been observed to breed both under winter and summer conditions in the Gaya district (It is taken for granted that the rainy season favours the breeding)

Observation 1, village Jamhor, December 1928 Breeding place, a cart-track about a few yards in length in main thoroughfare of the village fed by an over-flow sink This narrow trough of water was teeming with mosquito larvæ sufficiently to make the surface of the water look dark The larvæ were brought to the laboratory (9-12-28) and the imagines (about 40) which bred out of pupæ were all identified as *C fatigans* (14-12-28) Temperature on the above dates, dry and wet bulbs, 20-21 5°C, and 16-16 5°C, respectively

Observation 2, village Jamhor, 25th to 27th of May, 1929 Temperature dry and wet bulbs on the dates, 43°C and 23 5-24°C, respectively The interior of about 8 houses was visited The receptacles for storing water were found to be of earthen material The floor was found to be damp owing to spilling of water House drains leading from kitchens and bathing places contained water to the depth of 4 to 6 inches The drains were teeming with mosquito larvæ Mosquitoes sitting on the walls, and in the neighbourhood of the water storage, were in numbers and identified as *C fatigans* A large number of males were present and the probabilities were in favour of the fact that breeding was going on under the summer conditions Total collected 192, 37 males, all *C fatigans* and 2 *subpictus* Out of 143 *C fatigans* dissected, 10 showed developmental forms of *bancrofti* or 7 per cent

Observation 3, a similar picture to that of Jamhor was seen at Daudnagar (28-5-29) Total collected 131 (37 males), all *C fatigans*, 12 per cent of *C fatigans* showed developmental forms of *bancrofti*

The inference is that, in an endemic area [*vide* also (*b*) observation under urban area, Gaya town], owing to the perennial breeding of *C fatigans*, infection by *bancrofti* is kept up continuously

The following table, Table I, shows the results of dissection of Anopheline and Culicine mosquitoes in different months of the year in the belts of Bihar and Orissa

TABLE I

Showing percentage of infection in mosquitoes in different months of the year in the belts of Bihar and Orissa

Belt	Month	ANOPHELINES		CULICINES		
		Dissected	Positive	Dissected	Positive	Per cent
II	February			171	18	11
	March	15	0	272	21	8
	May	10	0	306	29	9
	June	2	0	93	13	14
	July	0	0	297	61	21
	August	43	0	170	7	4
	November			1	1	
	December	172	0	110	8	7
	TOTAL	242	0	1,420	158	11
III	January	347	1 ?	3	0	
	June	14	0	131	11	8
	July			89	9	10
	September	3	0	32	2	6
	December	268	0	7	0	
	TOTAL	632	1 ?	262	22	8
IV	January			101	12	12
	June			49	11	22
	July			300	18	6
	TOTAL			450	41	9

Judging from Table I, it appears that the Culicine mosquito shows a higher percentage of infection in the months of June and July in Belts II and III, in June in Belt IV

In Belt II, mosquitoes show a variable percentage of infection throughout the months of a year The data are insufficient to warrant any conclusions from Belts III and IV

Taking a census of the predominating species of mosquitoes, it was observed that the following species were found in the numerical order given *C fatigans* (528 males and 1,418 females), *A fuliginosus* (10 males and 625 females), *C whitmorei* (1 male and 202 females), *C vishnui* (25 males and 161 females), *A hyrcanus* (104 females), *A palludus* (7 males and 132 females) Other species of mosquitoes are not shown as their number was small

(f) Correlation between the incidence of infection by F bancrofti *in human host and in carrier* (C fatigans)

The data under (*b*), (*c*) and (*d*) when analysed show that, in Belt II, 1 urban, 7 suburban and 7 village areas were investigated In the urban area (total cases, 272, positive, 37) human infection is 13 6 per cent and mosquito infection (total dissected, 391, positive, 58) 14 8 per cent In the 7 suburban areas (total cases, 812, positive, 83) human infection is 10 2 per cent and mosquito infection (total dissected, 524, positive, 72) 13 7 per cent In the 7 village areas (cases, 636, positive, 62) human infection is 9 7 per cent and mosquito infection (dissected, 505, positive, 28) 5 5 per cent

In the Belt III, 1 urban and 9 village areas were investigated In the urban area, human infection (cases, 69, positive, 13) is 19 per cent and mosquito infection (dissected, 46, positive, 8) 17 per cent In the 9 village areas, human infection (cases, 627, positive, 4) is 0 63 per cent and the mosquito infection (dissected, 216, positive, 14) 6 4 per cent

In Belt IV, 2 urban and 5 village areas were investigated In 2 urban areas human infection (cases, 552, positive, 86) 15 5 per cent and mosquito infection (dissected, 356, positive, 37) 10 per cent In 5 village areas human infection (cases, 629 positive, 81) is 12 8 per cent and mosquito infection dissected, 94, positive, 4) 4 per cent

Speaking in a broad sense, the inference is that (1) there is some degree of correlation between human and mosquito infections in the urban areas, (2) there is no correlation in the village areas, and (3) there is suggestion that urban areas show a higher percentage of infection in human and mosquito population than the village areas

IV Discussion of results

The discussion resolves itself into two points —

(*i*) Does the prevalence of infection by *F bancrofti* vary in relation to terrain

In round figure, out of 6,176 cases examined in Bihar and Orissa 639 or 10 per cent showed microfilaria in the peripheral blood When the whole area is divided into belts, with due respect to physiography and physical characters the following percentages were obtained in each belt (Korke, 1927—29)

Belts I and II, Gangetic plain Belt I, total areas examined, 12, total cases, 292, positive, 22 or 8 per cent

Belt II, total areas examined, 36, total cases, 2,826, positive, 288 or 10 per cent

Belt III sub-montane and montane arable tract, total areas examined 20, total cases, 1,233, positive, 22 or about 2 per cent

Belt IV, sea coast belt total areas examined 12, total cases, 1,825, positive, 307 or 17 per cent

The data show that the sea coast belt is more infected than the Gangetic plain and the sub-montane and montane tract is practically free from infection

(*ii*) The nature of correlation between human and mosquito infection

The results show that there is no correlation in the mathematical sense between the degree of infection in human and mosquito hosts, except to a certain extent in the urban areas of the belts considered

V Conclusions

(1) That the infection by *F bancrofti* varies with the nature of the terrain It is highest in the sea coast belt, higher in the Gangetic plain and lowest in the sub-montane arable areas

(2) That the life cycle of *F bancrofti* has been observed in *Culex fatigans*, the domestic mosquito No other species of Culicine mosquito has so far been incriminated

(3) That the life cycle of *F bancrofti* has not been observed in the Anopheline species of mosquito

(4) There is no direct correlation between the *degree* of human and mosquito infections although there is such a suggestion in the urban areas

(5) *Culex fatigans* appears to be more infected in the months of June and July Incidence of infection appears to be higher when *C fatigans* is collected from the interior of houses

(6) Areas rich in paddy cultivation appear to be the endemic centres

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OBSERVATIONS ON THE CHARACTERS OF FILARIAL ENDEMIC AREAS IN BIHAR AND ORISSA

Part VI.

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It has been observed that the filarial situation in Bihar and Orissa is created by *F bancrofti* as the parasite and *C fatigans* as the vector and that the incidence of infection varies with the nature of the terrain (Korke, Part V)

From the preventive aspect it is instructive to classify the characters by which an endemic area is governed The endemic centres in Bihar and Orissa can be partitioned off into urban, suburban and village areas The main difference between an urban and suburban area is mostly one of density per square mile Both the areas are affected by some degree of sanitation

The villages are not protected by any measure of sanitation From the filarial standpoint there are roughly three types of villages to be considered The first type is a long straggling village situated along the highway, e g, the Grand Trunk Road Such a village has sprung up owing to traffic on the road and is known as 'Chatti', (Bihar area and Chota-Nagpur plateau) The second type is a 'huddled' village where houses are irregularly massed together The natural drainage from the houses falls into the tortuous bye-lanes in which water accumulates according to the quantity used and the season which allows it to remain or to evaporate, (arable areas in Bihar) The third type does not materially differ from the second type, except for the fact that, the groups of houses (thatched in most cases) appear to be isolated and hidden by thick growth of vegetation, and are supplied by an abundance of water from the shallow tanks and excavations, (Orissa area)

The correlated evidence of the Inquiry (1927—29) reveals two sets of facts in relation to terrain, first, the prevalence of filarial infection, secondly, the distribution of surgical signs of filariasis in relation The signs taken for analysis are the affections of genitals in the male, termed 'scrotal' and of

extremities termed 'terminal' An individual may have both signs present Other signs like chyluria (cases few) are not taken into the count Symptoms like the history of duration and of fever are omitted as the information relating to them is not exact The female population investigated was comparatively small in number, (total cases 191, positive 19)

Summary of Results

In the montane and sub-montane belt of Bihar and Orissa, total cases examined in 3 urban areas, 195 positive 16, percentage 8 2 Total cases in 25 village areas, 1,001, positive 6, percentage 0 6 Total cases convicts, from this belt, 37, positive nil

Distribution of surgical signs of filariasis, positive cases 22, showing signs, terminal 1 Negative cases 1,211, showing signs, scrotal 13, terminal 4, 1 and 0 3 per cent respectively

In the Gangetic belt, total cases examined in 3 urban areas 236, positive 30, percentage 12 7 Total cases in 9 suburban areas, 139, positive 50, percentage 11 4 Total cases in 37 village areas, 2,156, positive 205, percentage 9 5 Total convicts from this belt 287, positive 25, percentage 8 7

Distribution of surgical signs of filariasis, positive cases 310, showing signs, scrotal 58, terminal 5, 18 7 and 1 6 per cent respectively Positive cases not showing signs 249, 80 3 per cent Negative cases 2,808, showing signs, scrotal 312 terminal 69, 11 and 2 4 per cent respectively

In the sea coast belt, total cases examined in 3 urban areas 564, positive 90, percentage 16 Total cases in 15 village areas 1,113, positive 186, percentage 16 7 Total convicts from this belt, 148, positive 31, percentage 20 9

Distribution of surgical signs of filariasis, positive cases 307, showing signs, scrotal 43, terminal 12, 14 and 3 9 per cent respectively Positive cases not showing signs, 253, 82 4 per cent Negative cases 1,518, showing signs, scrotal 67, terminal 128 4 4 and 8 4 per cent respectively

Discussion of Results

In studying the prevalence of infection the points to be taken into consideration are (1) the distribution of infection in relation to terrain, and (2) the intensity in relation to Urban, Suburban and Village areas

1 Does the prevalence of filariasis vary with the nature of terrain? The answer is in the affirmative (Korke, Part V)

2 Which local type in an area is the primary seat of infection? The evidence shows that in a sub-montane arable area the general distribution of infection is negligible (0 6 per cent) but the intensity in urban areas is high, 8 2 per cent (in the town of Hazaribagh, the infection is 18 8 per cent) The urban area may therefore be presumed to be the starting point of infection The evidence in regard to the urban and suburban areas of the Gangetic

plain supports this presumption, since here also the village areas show a lower percentage of infection than the urban or suburban areas

In the sea coast area, both the village areas are equally affected with the urban areas and the evidence points to the fact that the physical characters of the area and the type of village are such as greatly to favour infection when once introduced

In all the belts studied, the physical value of the soil is such, as to yield a staple crop like paddy

3 When once introduced what factors can keep up continuously the infection? The factors are physical and biological The significant physical factor appears to be a soil retentive of moisture To support this view, I recall the evidence in connection with the Sone Canal investigation where the prevalence of filariasis increases from the upper to the lower reaches of the canal (Korke, 1929, Part III) The other evidence is that the prevalence decreases as one reaches an incline The observations refer to the investigation of the village areas on the Grand Trunk Road west to east From Aurangabad to Bhalua the road runs for about 60 miles through the Gaya plain Five areas equidistant on this section of the road show an infection, 7, 7, 13, 10 and 8 per cent respectively, (Korke, 1928, Part II and 1929, Part III) The second section of the road runs along the lower Hazaribagh plateau (where moisture conditions are unfavourable) for about 80 miles, from Bhalua to Topchanchi Four areas nearly equidistant on this section showed negative results From Topchanchi the road declines towards Calcutta, the infection at Topchanchi is 3 per cent The biological factor is the insect carrier So long as there is the prevalence of *C fatigans* and the opportunities are given to it to breed, feed and flourish, the endemicity of an area would be maintained

Regarding a further point in the investigation, that cases in the Gangetic plain show more signs referring to scrotal than terminal and vice versa in the coastal area, pathological research is indicated The only point of importance in this connection is that the terminal affections, at least in one of the areas of Orissa (Balasore district) are associated with the *atypical* forms of *bancrofti*

A large percentage of population harbour microfilaria without showing obvious signs and as to what happens to such cases in the long run, is a notable point for inquiry

To sum up the filarial situation in Bihar and Orissa the evidence shows that the prevalence of filariasis is greatest in the urban coastal area, where the value of the arable land is such as to yield a rich paddy cultivation This evidence has a special importance in a large country like India By studying the physical map of India, one may be able to foretell that filariasis should prevail predominantly in the areas like the Coromandel, Northern Circars, Konkan and Malabar Coasts (sea coast belt) less so in the Gangetic and the Indus plains which are physiographically on a higher level than the coastal belt

Conclusions

The factors which govern the characters of an *intense* endemic area of filariasis appear to be (1) terrain at the sea-level, (2) arable nature of land where physical factors are such as to yield a staple crop like paddy, (3) urban or suburban population, (4) incidence of *F bancrofti*, (5) presence of *Culex fatigans*, (6) collection of water under insanitary surroundings

In regard to surgical signs of filariasis cases in the Gangetic plain show more signs referring to scrotal than to terminal and vice versa in the coastal area

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OBSERVATIONS ON RAT-FLEAS AND THE TRANSMISSION OF PLAGUE

Part II

BY

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FLEA SURVEY OF BOMBAY CITY

IN connection with study of the flea factor in plague epidemiology it was decided to obtain more detailed information regarding the rat-flea population of Bombay city, especially concerning the regional distribution and the rodent hosts of the three species of *Xenopsylla*

Hirst (1926) notes that there is no striking irregularity in the distribution of human plague in Bombay, but that there is no record of the distribution of *cheopis* and *astia* in different parts of the city The Indian Plague Commission (1908) give data indicating the gross flea index of Bombay rats, of which an extract is shown in Table I All the rat-fleas were then classed as *Pulex cheopis* (now *X cheopis*) Attention was then drawn to the fact that *R norvegicus* harbours more fleas than *R rattus*, and one point of interest now is whether the three species of *Xenopsylla* are equally attracted to these two hosts Several more recent reports have given an indication of the relative prevalence of the three species in Bombay (Table II) These figures were the result of examining batches of fleas which had been collected without distinction of either host or area, and they give no indication of the flea index

TABLE I

Rat-flea index, Bombay city, 1906-07

Rodent	Average for year	Highest index	Lowest index
R rattus	4·0	5·2 (March)	2·5 (Nov.)
R norvegicus	8·4	13·9 (April)	4·2 (Sept.)

TABLE II

Proportion of Xenopsylla species, Bombay city

Period	Total examined	*cheopis*	Percentage of *astia*	*brasiliensis*
1920(3)	781	19·5	19·8	0·7
1922-23(4)	3075	58·1	15·8	1·0
1928(5)	1611	77·5	19·2	3·1

It was proposed to examine monthly samples from six areas. With the assistance of the municipal authorities, the following scheme proved satisfactory.—The rat-catching personnel selected suitable groups of buildings, and one day per month was allotted to each area. On the appointed morning a dozen traps, each containing only a single rat, were selected from a large number set overnight. These were put at once in stout canvas bags, securely tied, labelled and sent to the laboratory. There they were put in large tins, the bags were opened up, and a pad soaked in petrol or chloroform was introduced to the tin. In twenty minutes rats and fleas were dead, and the contents of the tins could be searched for fleas.

It is necessary to emphasize the fact that vigorous thumping of the carcase on a hard surface, and with several grips, is essential, in addition to the usual combing and brushing, if all the fleas are to be obtained. In searching for the fleas a wooden trough, five feet by three feet, and three inches deep, covered with white oil cloth, is satisfactory, and it is convenient to have this constructed on a table of suitable height for the worker to stand in comfort, say four feet.

The rats received were classed as follows.—(1) *Rattus rattus*, including white-bellied, black and brown varieties. These are easily recognized by the length of the tail which is often about one and a quarter times as long as head and body combined, although it may on occasion be much the same length. If the tail has been amputated, the large ears and eyes and the narrow muzzle are simple points for identification. Hinton(6) refers to the impossibility of dealing with varieties of the house rat in ports like Bombay, where indigenous rats have interbred with old wanderers and recent newcomers from all parts of the world.

(2) *R. norvegicus*, with a brush-tipped tail distinctly shorter than head and body together, small ears and eyes, and large flesh-coloured feet.

(3) Gunomys sp. mole rats, differing from *norvegicus* in the broad head, dark feet with small round pads, and a tapered tail which lacks a brush at the tip *

* The Bombay Gunomys was formerly familiar under the name of *Nesocia bengalensis* Oldfield Thomas (quoted by Lloyd 1909, *Records Indian Museum*, III 1 12), distinguished Gunomys, with a long palatine foramen (8 mm.) and a tail averaging 80 per cent. of the head and body length from *Nesocia* with a short palatine foramen (5 mm.) and a short tail only 50 per cent. of the head and body length. According to Wroughton (1919, *Jour. Bomb. Nat. Hist. Soc.*, XXVI. 3. 787), the common species in Western India is *G. kok*, the southern mole-rat.

The three groups can also be distinguished, when alive, by eliciting the characteristic noise *R rattus* gives a series of squeaks, *norvegicus* a prolonged squeal, while Gunomys grunts No bandicoots or mice were caught, the traps being unsuitable for these

The areas selected for the survey are briefly described in Table III With the exception of Mazagaon and Worli, which are residential, the buildings included bazaar shops as well as habitations

TABLE III

Areas selected for flea survey of 1929-30

No	Ward	Address	Details	Building	Occupants
1	A	Fort	Bora Bazaar St W Side	Large tenements	Parsee Hindu
2	Ee	Mazagaon	Rose Cottage Lane W of Jumna Flour Mill	One-storied	Chiefly Hindu
3	C	Bhuleshwar	Between Sandhurst Rd and Durgadevi St	Large tenements	Hindu
4	D	Chikalwadi	Between Slater Rd and Tardeo Rd	Large tenements	Hindu
5	G	Worli	Village	One-storied Mud walled	Christian Hindu
6	G	Mahim	S E corner of bazaar	Two-storied	Mohammedan

With regard to the rodents (Table IV), the absence of *norvegicus* in Worli village, along with the absence of a drainage system, was previously noted by

TABLE IV

Rodents examined in the course of the flea survey

Area	*R rattus*	*R norvegicus*	Gunomys	Total
1	91	43	10	144
2	134	3	8	145
3	102	20	22	144
4	79	29	33	141
5	144			144
6	132		12	144
TOTAL	682	95	85	862
Females	407	60	46	
Males	275	35	39	
Rats with no fleas	88	11	9	

the Plague Commission No *norvegicus* was received from Mahim These two areas are the most remote from the influence of shipping The presence of Gunomys, even in densely populated quarters of the city, is of interest, this animal being by nature a ferocious field rat It has apparently adapted itself to domestic surroundings and must be in close contact with man in these places The number of rodents which harbour no fleas at all is over 10 per cent in the three groups This fact would have been missed if the investigation had not been confined to rats caught singly

The gross flea index (Table V) shows that, compared with *rattus*, the number of fleas harboured by *norvegicus* is more than half as many again, while

TABLE V

Identification of the fleas according to the host

Fleas	*R rattus*	*R norvegicus*	Gunomys
All species—			
Number	2,988	655	816
Index	4·1	6·9	9·6
X cheopis—			
Number	2,499	420	186
Percentage of total	83·6	64·1	22·8
Index	3·7	4·1	2·2
X astia—			
Number	350	233	621
Percentage of total	11·7	35·6	76·1
Index	0·5	2·5	7·3
X brasiliensis—			
Number	137	2	6
Percentage of total	4·6	0·3	0·7
Index	0·2	0·0	0·1
Ctenocephalus—			
Number	2	0	3

Gunomys harbours more than twice as many fleas as *rattus* Referring to the specific flea index, it is seen that the increase in both cases is due chiefly to the greater number of *astia* Although *norvegicus* has only 19 per cent more

cheopis, it has five times as many *astia* Gunomys shows 40 5 per cent fewer *cheopis* but more than fourteen times as many *astia*

The record number of fleas found on individual rodents may be mentioned viz, *rattus* 27 (14 *cheopis*, 5 *astia*, 8 *brasiliensis*), *norvegicus* 90 (47 *cheopis*, 43 *astia*), Gunomys 74 (2 *cheopis*, 71 *astia*, 1 *brasiliensis*) The *brasiliensis* index is very small throughout, but the species has actually been found in each of the three groups of rodents The only fleas, other than *Xenopsylla*, were five *Ctenocephalus felis*, two on *rattus*, three on Gunomys

The *Xenopsylla* are analysed according to species and sex in Table VI The same variation in the proportion of the sexes of *cheopis* and *astia* was

TABLE VI

Xenopsylla *according to species and sex*

	X cheopis		*X astia*		*X brasiliensis*	
	Female	Male	Female	Male	Female	Male
Number	1,237	1,868	734	470	50	95
Percentage of sexes	39·8	60 2	61 0	39·0	34 5	65 5
Percentage of species	69·6		27 0		3 3	

shown in one of the earlier surveys, (Webster, 1929) viz, *cheopis* females practically 40 per cent, *astia* females over 60 per cent This difference is not easily explained as breeding experiments do not show a similar variation The small *brasiliensis* numbers indicate a preponderance of males As the sex of the fleas is a factor in plague transmission, this variation in the proportion of the sexes in wild fleas may be of some importance

Reference to Table VII indicates that the specific flea index of *R rattus* is not subject to extreme variation throughout the year The *cheopis* index is within the limits of 2 2 and 5 4, while the *astia* average is constantly less than one per rat Taking the areas separately (Table VIII), the lowest gross flea index (2 4) is from the area which is in a distinctly better class neighbourhood, while the highest index (6 0) belongs to the primitive village of Worli The only area yielding *brasiliensis* in appreciable numbers is Mahim This is situated on the border of a dense palm forest The local conditions are cooler and damper than in the other areas, and it is the only one of the six with a considerable amount of rural vegetation

There is, therefore, every indication that *cheopis* and *astia* are both widely distributed in Bombay In no area is there evidence of a constantly high *astia* or *brasiliensis* index

TABLE VII

R rattus *by months*

Month	Rats	Fleas	Flea index		
			X cheopis	*X astia*	*X brasiliensis*
March	58	290	3·1	0·5	1·1
April	56	275	1·1	0·5	0·1
May	51	263	3·9	0·7	0·3
June	58	110	2·2	0·2	0·0
July	65	351	1·8	0·6	0·0
August	58	272	1·2	0·5	0·0
September	65	243	3·0	0·5	0·2
October	43	219	5·1	0·1	0·0
November	58	231	3·1	0·3	0·6
December	54	160	2·3	0·7	0·0
January	61	242	3·1	0·5	0·0
February	53	269	1·3	0·8	0·0
TOTAL	682	2988	3·7	0·5	0·2

TABLE VIII

R rattus *by areas*

Area	Rats	Fleas	Flea index		
			X cheopis	*X astia*	*X brasiliensis*
1	91	217	1·9	0·4	0·1
2	134	700	4·8	0·4	0·0
3	102	336	2·9	0·4	0·0
4	79	277	3·1	0·4	0·0
5	144	868	5·3	0·7	0·0
6	132	590	2·9	0·6	1·0

Details for *norvegicus* and Gunomys (Tables IX—XII) deal with comparatively small numbers, but they confirm the fact that the high flea index of

these rodents, as compared with that of *rattus*, is largely due to a higher infestation with *astia*

TABLE IX

R norvegicus *by months*

Month	Rats	Fleas	Flea index		
			X cheopis	*X astia*	*X brasiliensis*
March	6	51	7·3	1·2	0·0
April	10	81	5·7	2·4	0·0
May	9	30	2·6	0·8	0·0
June	8	138	10·25	7·0	0·0
July	4	19	4·75	0·0	0·0
August	8	44	4·25	1·25	0·0
September	4	47	5·75	6·0	0·0
October	16	111	3·0	4·0	0·0
November	8	39	2·4	2·5	0·0
December	13	34	1·5	1·1	0·0
January	2	24	10·0	2·0	0·0
February	7	37	4·3	0·7	0·3
TOTAL	95	655	4·4	2·5	0·0

TABLE X

R norvegicus *by areas*

Area	Rats	Fleas	Flea index		
			X cheopis	*X astia*	*X brasiliensis*
1	43	248	4·2	1·5	0·0
2	3	27	8·3	0·7	0·0
3	20	149	4·5	3·0	0·0
4	29	231	4·3	3·6	0·0

TABLE XI

Gunomys *by months*

Month	Rats	Fleas	Flea index		
			X cheopis	*X astia*	*X brasiliensis*
March	5	19	5·0	1·6	0·0
April	7	215	1·7	28·7	0·3
May	9	92	2·5	7·6	0·1
June	6	29	0·6	1·2	0·0
July	3	28	2·3	7·0	0·0
August	7	67	1·7	7·1	0·1
September	3	19	1·0	2·3	0·0
October	13	51	1·0	2·9	0·0
November	6	65	1·0	9·8	0·0
December	5	37	2·8	4·6	0·0
January	9	67	2·3	5·1	0·0
February	12	97	3·1	4·8	0·0
TOTAL	85	816	2·2	7·3	0·1

TABLE XII

Gunomys *by months*

Area	Rats	Fleas	Flea index		
			X cheopis	*X astia*	*X brasiliensis*
1	10	45	1·0	3·3	0·0
2	8	111	3·25	10·6	0·0
3	22	170	2·4	5·3	0·0
4	33	313	2·6	6·9	0·0
6	12	177	1·1	13·25	0·4

ACKNOWLEDGMENTS

Dr Sandilands, late Executive Health Officer, kindly afforded facilities for the initiation of this enquiry, and his staff have rendered every assistance

SUMMARY

(1) A flea survey of Bombay city for the year commencing March 1929 is reported in detail

(2) *X cheopis* and *X astia* are both widely distributed, the former being five to twelve times more numerous on *R rattus* *X brasiliensis* is practically confined to the most rural area of the six which were studied No marked seasonal variation has been demonstrated Identification of 4,459 fleas gives the following percentages —*cheopis* 69 6, *astia* 27 0, *brasiliensis* 3 3, Ctenocephalus 0 1

(3) The gross flea index of the rodents examined is found to be — *R rattus* 4 4, *R norvegicus* 6 9, Gunomys 9 6 The three species of Xenopsylla have been found on each of these hosts

(4) The *cheopis* index is slightly higher for *norvegicus* than for *rattus*, while Gunomys harbours fewer *cheopis* than either

(5) The *astia* index for *norvegicus* is five times greater than for *rattus*, while for Gunomys it is fourteen times greater than for *rattus*

(6) The proportion of the sexes shows a marked difference in the three species of Xenopsylla The approximate percentage of females is —*cheopis* 40, *astia* 60, *brasiliensis* 35

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THE BLOOD-MEAL OF SANDFLIES INVESTIGATED BY MEANS OF PRECIPITIN ANTISERA

BY

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INTRODUCTORY

A FEW years ago the present writers with Dr R O A Smith carried out a number of observations on the blood-meal of sandflies, *Phlebotomus argentipes* In a paper (Lloyd, Napier and Smith, 1925) reporting our findings we discussed the possible bearing of our observations on the kala-azar transmission problem Our conclusions were shortly as follows —

That in Calcutta at the time of year during which the observations were carried out (November and December) *Phlebotomus argentipes* fed almost exclusively on the blood of man or cattle, that they preferred cattle to man, that other conditions being the same the presence of cattle afforded some protection to man against the bites of sandflies

The observations herein reported were carried out with the object of supplementing our earlier ones, they were extended over a period of two years, they were made on a much larger number of flies of three different species collected at different seasons from several different areas and from several different sites within those areas

The sandflies were collected, dissected and numbered by the junior writer or one of his insect collectors They were then sent to the laboratory of the senior writer to be tested, the latter had no knowledge of the origin of the specimens which he was testing The results of the precipitin tests together with full details of the source of each specimen were recorded at the time, but no attempt at analysis of the record was made until all the figures had been

TABLE II.

	Phlebotomus from all sources			Culicoides (mostly peregrinus).	Phlebotomus from site C	
	argentipes I	minutus (babu) II	papatasii III	IV	argentipes V	minutus VI
Total number of specimens tested	909	110	31	11	27	95
" " in which both human and bovine blood were detected	258	16	4	1	3	16
" " " " human blood alone was detected	25	16	1	0	0	10
" " " " bovine " " " "	586	45	29	9	23	39
" " " " neither human nor bovine blood was detected	40	33	0	4	1	30
Percentage of the total in which either human or bovine blood was detected	95·6	70	(100)	(71·1)	(96·3)	68·4
" in which human blood was detected	31·1	29·1	(14·7)	(7·1)	(11·1)	27·4
" " " bovine " " "	92·8	55·5	(97·1)	(71·4)	(96·3)	57·9

Note —The percentages shown within brackets being calculated from small series have only a provisional value

B The feeding habits of flies caught during different months of the year

Table III gives details of the feeding habits of the flies caught at the Kaorapukur kala-azar treatment centre month by month —

TABLE III

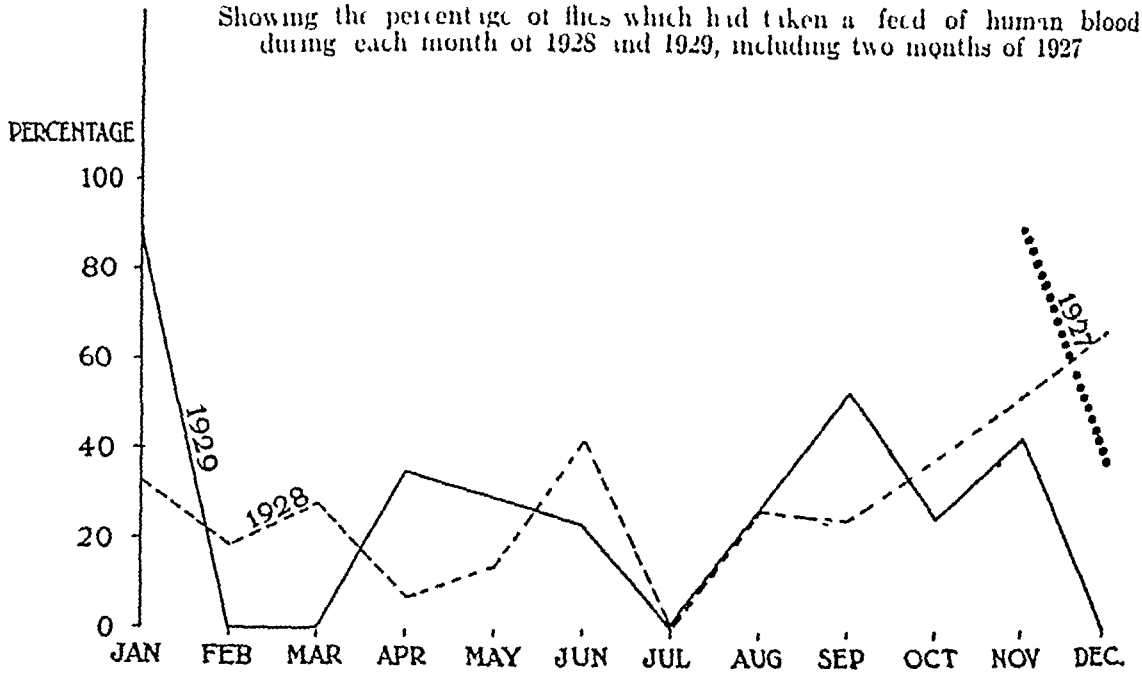
Phlebotomus argentipes *collected in the Kaorapukur kala-azar treatment centre area month by month*

Month	Total number of flies tested	Number in which both human and bovine blood were detected	Number in which human blood alone was detected	Number in which bovine blood alone was detected	Number in which neither human nor bovine blood was detected	Percentage of human feeds
November 1927	30	26	1	3	0	90·0
December „	38	14	0	24	0	36·8
January 1928	135	42	2	88	3	32·6
February „	97	16	2	72	7	18·6
March „	97	22	5	66	4	27·8
April „	72	5	0	65	2	7·0
May „	36	4	1	31	0	14·0
June „	33	14	0	15	4	42·4
July „	8	0	0	8	0	0
August „	41	11	0	30	0	26·8
September „	20	3	2	15	0	25·0
October „						
November „						
December „	18	12	0	6	0	66·7
January 1929	8	7	0	1	0	87·5
February „	9	0	0	9	0	0
March „	8	0	0	8	0	0
April „	8	3	0	4	1	37·5
May „						
June „	22	5	0	16	1	22·7
July „	25	0	0	25	0	0
August „						
September „	19	9	1	8	1	52·6
October „	4	1	0	3	0	25·0
November „	37	16	0	18	3	43·2
December „	21	0	0	17	4	0
TOTAL	786	210	14	532	30	

The percentage of flies which take a feed of human blood has been calculated for each month and shown in the right hand column of the above table The percentages are shown below in graphic form (Graph 1) for the two years 1928 and 1929 separately

GRAPH 1

Showing the percentage of flies which had taken a feed of human blood during each month of 1928 and 1929, including two months of 1927



The curves for the two years have certain features in common At the beginning of the year there is a fall followed by a rise as the hot weather advances, in 1928 the maximum occurred in June and in 1929 in April, but as in the latter month there were only 8 flies caught, chance sampling may have played an important part There is a definite fall in July, again the numbers in 1928 were small, but in 1929 they were much larger and included flies from several sites There is a second rise in the autumn, in 1927 and 1929 a fall again occurred in December but in 1928 the rise continued into January Graph 2 shows the combined figures for the two years 1928 and 1929, October, during which only 4 observations were made, is omitted from the graph

As the flies on which the graphs are based were caught from several different sites, mostly cowsheds, but situated at varying distances from human sleeping quarters, a separate analysis was made of the results of the blood-meals of the flies caught from one site on different dates Two hundred and sixty-nine *P argentipes* were caught from site G, a cowshed situated 15 yards from a house in the endemic area occupied by a number of persons Table IV summarizes the blood-meals of these flies during each month of the year, and these figures are shown on Graph 3, again October has been omitted as there were only 4 flies dissected in that month The curve shows much the same features as the others, viz , a rise in June and a second rise in the autumn

GRAPH 2

Showing the percentage of flies which had taken a feed of human blood during each month (Combined figures for 1928 and 1929)

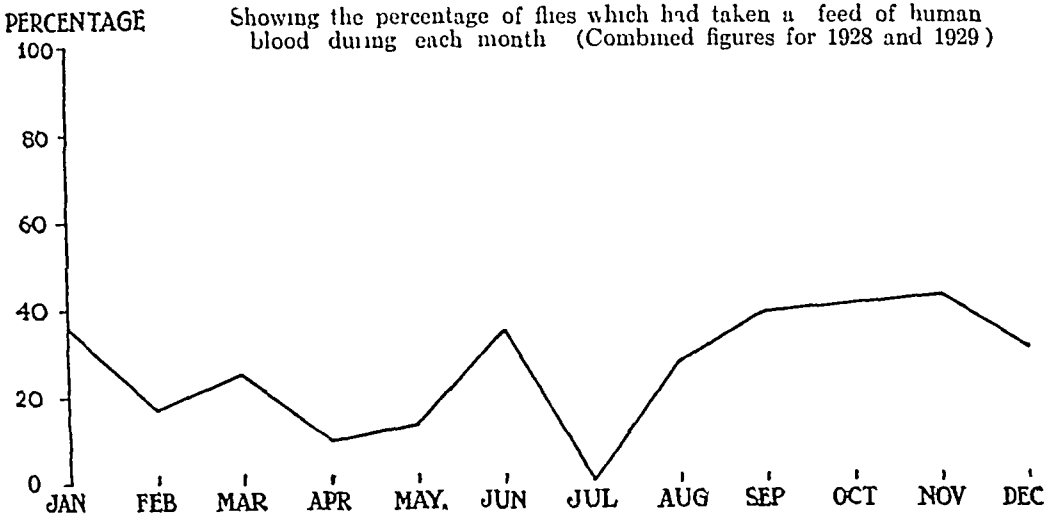


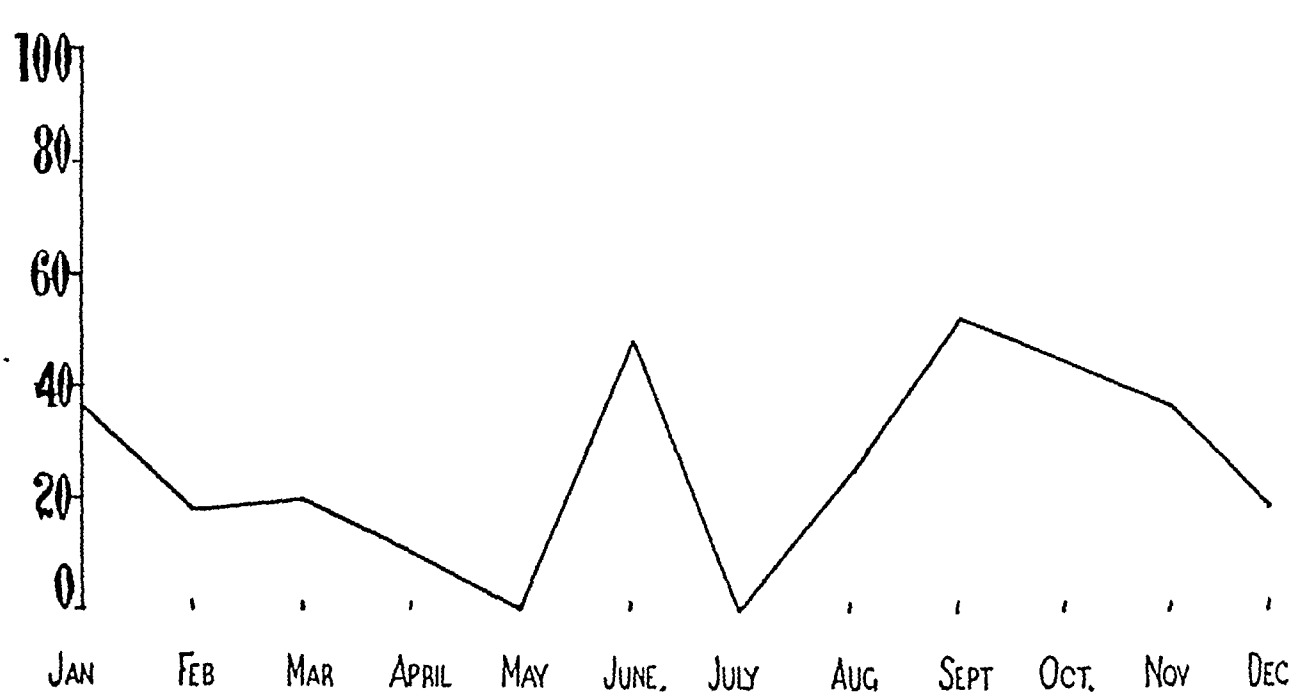
TABLE IV

P argentipes *caught at one site* (G) *during different months of the year* (*composite table for* 1928 *and* 1929)

Month	Total number of flies tested	Number in which both human and bovine blood were detected	Number in which human blood alone was detected	Number in which bovine blood alone was detected	Number in which neither human nor bovine blood was detected	Percentage of human feeds
January	30	11	0	19	0	36 6
February	11	2	0	9	0	18 2
March	30	6	0	23	1	20·0
April	28	3	0	24	1	10·7
May	16	0	0	16	0	0
June	27	13	0	12	2	48 1
July	23	0	0	23	0	0
August	35	9	0	26	0	25 7
September	19	9	1	8	1	52·6
October	4	1	0	3	0	25 0
November	19	7	0	10	2	36 8
December	27	5	0	19	3	18·5
TOTAL	269					

GRAPH 3

Showing the percentage of flies from site G which had taken a feed of human blood during each month of the year (Composite graph for 1928 and 1929)



The seasonal variation in the feeding habits of these sandflies requires some explanation It is not difficult to explain the autumn rise in the percentage of human-blood-feeding flies In order to obtain the strongest flies in the laboratory we breed them under monsoon conditions of temperature and humidity Flies caught during the months of August, September, October and November have been bred under these conditions and are, consequently, stronger, longer lived and able to fly further from the cowshed for their blood-meal They therefore have a greater opportunity of getting a human blood-meal at some time during their life But the sharp rise in June is difficult to explain

C Differences in the feeding habits of sandflies in different localities

Most of the flies here analysed were caught in the Kaorapukur area near Calcutta, a few however were caught in another rural area near but on the other side of Calcutta, also a kala-azar endemic area Others were caught at Purulia, a non-endemic area 200 miles from Calcutta situated on the lower slopes of the Bihar plateau about a thousand feet above sea-level

P argentipes were found in Purulia, but in small numbers except during the monsoon and the period immediately after Starting with the premise that this sandfly transmits kala-azar, our object in examining flies from this area was to see if any difference in the feeding habits of the flies could be established

which would account for the fact that kala-azar was not endemic in this area We naturally expected to find that the flies in Purulia fed less frequently on man than the flies in the endemic area, whereas we actually found the contrary to be the case

In Table V below the results of the examination of the blood-meals of 71 flies caught at Purulia and 92 flies caught during the same months in an endemic area in Bengal are summarized —

TABLE V

	Endemic area	Purulia (non-endemic area)
Total number of specimens tested	92	71
" " in which human and bovine blood were detected	33	35
" " in which human blood alone was detected	0	4
" " in which bovine blood alone was detected	58	24
" " in which neither human nor bovine blood was detected	1	8
Percentage of total in which either human or bovine blood was detected	98·9	88·7
" in which human blood was detected	35 9	54 9
" in which bovine blood was detected	98 9	83 1

D Differences in the feeding habits of sandflies, P argentipes, *caught at different sites in the endemic area*

Unfortunately nearly all the flies used in this investigation came from cowsheds In human habitations not only are the flies fewer in number and more difficult to catch, but there are certain difficulties in obtaining admission to private dwellings, which do not arise in the case of cowsheds From one site, B_1, a living room situated 25 yards from a cowshed, 16 flies were caught, and 15 from another site, B_2, an empty room situated about 25 yards from

a cowshed on one side and from human sleeping quarters on the other The results of analysis of the blood-meals are given below (Table VI) —

TABLE VI

Site	B_1	B_2
Total number of specimens tested	16	15
" " in which both human and bovine blood were detected	2	5
" " in which human blood alone was detected	6	0
" " in which bovine blood alone was detected	7	10
" " in which neither human nor bovine blood was detected	1	0
Percentage of total in which either human or bovine blood was detected	93·75	100·0
" in which human blood was detected	50·0	33·3
" in which bovine blood was detected	56·25	100·0

We have seen above (Table II) that of the sandflies (*P argentipes*) obtained from all sources, mostly from cowsheds, 92 8 per cent contained bovine blood and 31 1 human blood, whereas we observe from Table VI that of *P argentipes* caught from human sleeping quarters only 50 per cent contained human blood and 56 25 per cent bovine blood This certainly adds support to the suggestion previously made that *P argentipes* feeds more readily on bovine than on human blood Furthermore, when both kinds of blood are available at some little distance (2nd column of Table VI) all the flies took the bovine blood, but only one-third took human blood

E The influence of distance on the feeding habits of the sandflies

The cowsheds in which flies were caught were situated at different distances from the human habitations In the endemic area there were altogether 8 sites from each of which more than 25 flies were caught and examined The number of flies caught in each site, the percentage of these containing human blood

and the distance of the site from the nearest human habitation are given in Table VII —

TABLE VII

Site	Distance of site from human sleeping quarters	Number of flies examined	Percentage in which human blood was detected
I	Adjoining	29	34·5
P	„	35	31·4
H	6 yards	82	30·5
W	12 yards	27	22·2
G	15 yards	269	24·9
K	20 yards	62	14·5
B	30 yards *	58 *	19·0 *
C	35 yards *	42 *	35·7 *
B excluding one catch	*	50 *	6·0 *
C excluding one catch	*	27 *	11·1 *

* *See* note

Note—Sites B and C are large cowsheds some distance from any human sleeping quarters, but it was ascertained that occasionally men slept in the shed with the cattle A total of 58 flies was caught on 10 occasions from site B, on one occasion 8 flies were caught and all were found to contain human blood, whereas of the remaining 50 from site B only 6 per cent contained human blood In the case of site C out of a total of 42 flies one particular catch consisted of 15 flies of which 12 contained human blood, whereas of the remaining 27 from site C only 11 1 per cent contained human blood It would thus appear to be obvious that these two catches, one from each site, must have been occasions when men were sleeping in the sheds

It will be seen from Table VII that, after making the allowances explained in the note attached thereto, the percentage of human feeds is in inverse ratio to the distance from human sleeping quarters of the site where the flies were caught This is, of course, what would be expected

In the investigation reported in our previous paper *P argentipes* were caught in the urban areas of Calcutta Here large numbers of flies were caught in houses where no cows were kept, the nitrogenous matter which the flies require in their larval stage being provided by refuse and the droppings of other animals Such flies almost always contained human blood When, however, there were cows in the vicinity, it was found that the great majority of the flies contained bovine blood alone It was therefore concluded that the

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Jour London School Trop Med
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ON THE VIBRIOCIDAL POWER OF THE WATER OF CERTAIN RIVERS OF INDIA

BY

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(From the Bacteriophage Inquiry, Indian Research Fund Association, Patna)

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It is a well-known fact that the *V. cholerae* dies out quickly when added to water. A good deal of experimental work has been done outside India which need not be referred to here. All this work proves the above-stated fact. Hankin (1896) added cholera vibrios to the water of the rivers Jumna and Ganges and found that they soon died out. Greig's work in India corroborated those findings (d'Herelle, 1929). We (Khan and Agarwal, 1929) found that in the Ganges and the Jumna river water the vibrio died out in almost 24 hours. In the same water, only boiled, it lived on the average for about 3 days. Hankin noticed this loss of the vibriocidal power after boiling. He stated that the vibriocidal power of the water of the rivers Jumna and the Ganges was due to a volatile substance which volatilized on boiling. He, however, adduced no experimental evidence in proof of this view. Now, d'Herelle (1926) referring to Hankin's work put in his own phenomenon in explanation of this fact. He stated that 'the vibriocidal action observed by Hankin must necessarily be referred to bacteriophagy'.

After our work on the duration of the life of vibrios in the Ganges and the Jumna river water, it was found desirable to investigate the cause of the vibriocidal power of the water of these rivers. If it be true in nature, as it may be in the laboratory, that waters which contain bacteriophage are inimical to the cholera vibrio, and those waters which do not contain this principle are not, then certainly this fact is of much practical importance in the epidemiology of cholera. For it then seems quite logical to suppose that the addition of bacteriophage to natural sources of water may be expected to render them safe with regard to past or future contamination with the cholera germs. We, therefore, took a sample of water from the Ganges at Patna in order to see if its vibriocidal power was due to a volatile substance or to bacteriophage.

TABLE

	Date	Raw Ganges water	Boiled water 5 minutes open boiling	Boiled water in sealed test-tubes for 5 minutes	Filtered water through Chamberland candle L3	Water heated at 55°C for half an hour	Distilled Ganges water	Open boiled water plus vapour from raw water at 60°C for 15 mins	Open boiled water plus vapour from raw water at 70°C for 15 mins	Open boiled water plus vapour from raw water at 80°C for 15 mins	Open boiled water plus vapour from raw water at 90°C for 15 mins	Water heated at 50°C for 15 mins	Water heated at 60°C for 15 mins	Water heated at 70°C for 15 mins	Water heated at 80°C for 15 mins	Water heated at 90°C for 15 mins	Raw Ganges water
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Milligrams of *Vibrio cholerae* 60 added on	1-5-29	0 222	0 222	0 222	0 222	0 222	0 222	0 222	0 222	0 222	0 222	0 222	0 222	0 222	0 222	0 222	Nil
Number of vibrionic colonies on	2-5-29	0	128	25	4	119	0	250	165	130	121	0	105	159	121	109	0
"	3-5-29	0	100	25	0	101	0	102	100	111	2	0	210	311	172	142	0
"	4-5-29	0	0	0	0	0	0	1	0	0	0	0	2	110	106	100	0
"	5-5-29	0	0	0	0	0	0	0	0	0	2	0	1	1	3	0	0
"	6-5-29	0	0	0	0	0	0	0	0	3	1	0	0	0	0	2	0
"	7-5-29	0	0	0	0	0	0	0	0	0	100	0	0	0	0	2	0
"	8-5-29	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0
"	9-5-29	0	0	0	0	0	0	0	0	0	102	0	0	0	0	0	0
"	10-5-29	0	0	0	0	0	0	0	0	0	135	0	0	0	0	1	0
"	11-5-29	0	0	0	0	0	0	0	0	0			Not	plated	out		
"	12-5-29	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0
"	13-5-29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
"	14-5-29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total number of vibrionic colonies		0	228	50	4	220	0	353	265	247	663	0	318	587	405	356	0

The protocol of the experiment is given at the end of this paper The reaction of the water was rather alkaline being pH 8 at that time of the year (30th April, 1929) This water was divided into several portions of about 100 c c each and placed in Ehrlenmeyer's flasks of 200 c c capacity Seven of these portions were examined for the presence of a cholera bacteriophage and it was found that none of them had any such bacteriophage The technique for the detection of choleraphage was as follows To each hundred cubic centimetres of the water enough concentrated broth was added to make the whole equal in strength to the ordinary broth Then a cholera culture was taken which experience had shown to be the most susceptible to the action of bacteriophage About 3 milligrams (by Brown's opacity method) of this culture were then added to the water The water thus treated was left at room temperature and filtered next morning through a Chamberland candle L3 Ten cubic centimetres of this filtrate were added to a fresh emulsion of the cholera vibrio in 10 c c of broth and were found to contain no choleraphage—there was no lysis at all and when 0 04 c c of the mixture was plated out on a Petri dish and the plate incubated at 37°C for 24 hours, no plaque of bacteriophage was discovered

This part of the experiment showed that the sample of the water contained no choleraphage At least none was discovered by a reasonably intensive method for the detection of bacteriophage

The vibriocidal power throughout this experiment was determined as follows To a flask containing 100 c c of the Ganges water about 0 222 milligrams of a 24-hour old culture of cholera vibrio was added One cubic centimetre of this water was inoculated into 8–10 c c of peptone water the reaction of which was pH 8 and after incubating for four hours at 37°C, an ordinary-sized loopful was plated out on a bile salt agar plate The plates were examined after 18 hours of incubation, and the number of vibrionic colonies counted For further confirmation some of these colonies were tested for agglutination, and also for morphology by staining If no vibrio could be recovered from the water by this technique for a number of successive days, it was presumed that the vibrios that were added had died out

In spite of the fact that the water contained no choleraphage as described above, its vibriocidal power had not suffered in the slightest degree The vibrios that were added to the raw Ganges water died out in less than 24 hours The vibriocidal power of the water of the Ganges, therefore, is not due to bacteriophage

A part of the rest of the experiment was devised to see if the vibriocidal power was due to a volatile *substance* In the first place, therefore, one hundred cubic centimetres of the water were boiled for five minutes in sealed test-tubes No volatile substance could have been lost to any appreciable degree under these circumstances, yet the vibriocidal power was lost considerably In the water boiled in sealed test-tubes the vibrio lived for 2 days Secondly, a little of the water was placed in flask A, which was connected by means of a glass tubing with an Ehrlenmeyer flask B containing 100 c c of

the water which had been previously boiled for 5 minutes Flask A was heated at 60°C for 15 minutes in order to volatilize any volatile substance from flask A to flask B Similarly it was tried to volatilize the *substance* at 70°C into another flask C containing 100 c c of boiled water and so on at 80°C into flask D and at 90°C into flask E In none of the flasks B, C, D and E did the boiled water regain its vibriocidal power by anything that might have volatilized into it from the raw water in flask A The vibrio lived in them for the normal period of three days

Nevertheless the water had lost its vibriocidal power in every case when heated for 15 minutes at 60°C, 70°C, 80°C or 90°C The vibrio lived in each of them for the normal period of three days Also, when the water was heated for half an hour at 55°C, it completely lost its vibriocidal power It, however, did not lose the vibriocidal power when heated at 50°C for 15 minutes

To what then is this vibriocidal power due? It is not, as Hankin supposed, due to a volatile substance in the water No vibriocidal substance can be volatilized over from the water and the water loses its vibriocidal power when heated under conditions that no volatile substance would escape

The vibriocidal power also is not due to bacteriophage The water was found to be just as vibriocidal even when no cholera phage could be isolated from it Further, the vibriocidal power was completely lost when the water was heated at such a low temperature as 55°C This temperature is too low to destroy any bacteriophage if it were present

It may, however, be suggested that the vibriocidal power depends on two vital principles of biology on which the duration of life of all living beings depends These are, firstly, the supply of food available for assimilation and, secondly, the presence of the products of metabolism In raw water there are many other organisms which are competitors of the available food material They use up the food present in the water and by so doing cause the death of the vibrios that are added It is possible the products of their metabolism may contribute in bringing about this result By heating the water at 55°C for half an hour most of these organisms are killed They, therefore, are now not only no longer competitors of food, but their dead bodies furnish food material for the *V choleræ* The *V choleræ*, therefore, lives for a much longer period in the heated water

Thus, if we filter the raw water through a Chamberland candle in such a way that *all the organisms present in the water are held back*, we will find that the filtrate which is free of all bacteria is still vibriocidal but not so much as raw water What is the explanation of this? It is possible that the vibrio lives longer in the filtrate than in raw water because the former is free of the extraneous organisms On the other hand it lives for a much shorter time in the filtrate than in boiled water because probably the filtrate is less rich in food material It certainly does not contain the dead bodies of most of the bacteria and other coarse organic matter that do not pass through a Chamberland candle L3

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SOME PECULIARITIES IN THE MALARIAL TEMPERATURE CHARTS OF CHITTAGONG HILL TRACTS

BY

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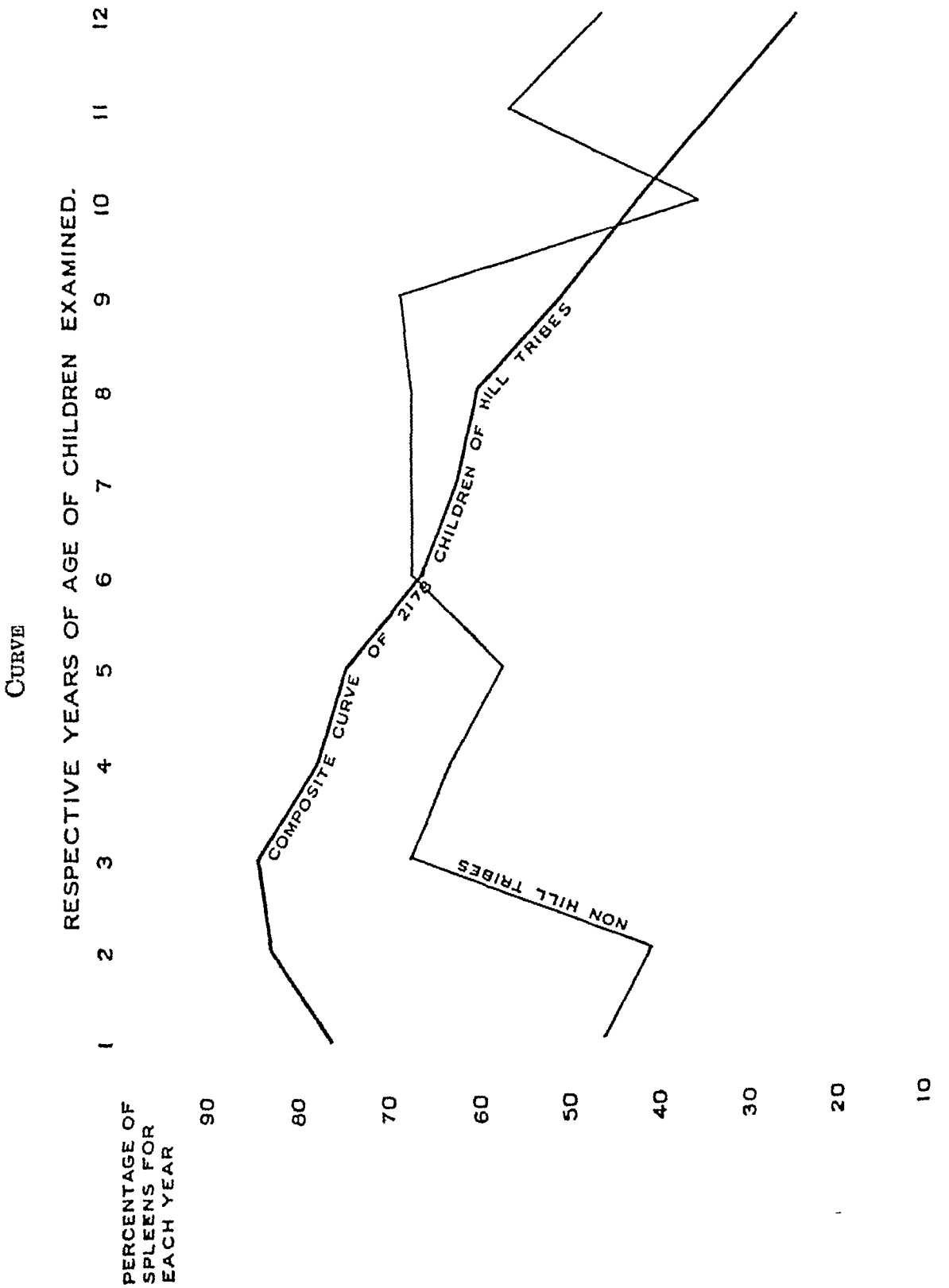
WHILE I was at Arambagh, a very malarious and unhealthy town in the district of Hooghly, I made a thorough spleen census of the children in the town and published my results in the form of a paper in the *Indian Medical Gazette* of September 1913 In this paper I showed that though there was no difference in the intensity of malaria as judged by the splenic census of the different wards of the municipality, still, when I tried to determine the amount of malarial infection in the different sub-castes of the Hindus, it was found that the members of some Hindu sub-castes scattered through the town who were residing from a very long time showed less malarial infection comparatively than other Hindu sub-castes who were new-comers Next when I was transferred to Nadia District, I published a paper named 'Some Studies in Malaria in Nadia District' in the *Indian Medical Gazette* of April 1916 At the time of collecting materials for this paper it came to my notice that repeated infections during early life leave a pronounced resistance against malaria in adult age

I was posted soon after to the Chittagong Hill Tracts as the Civil Surgeon of the place There I found that though splenic enlargement was very common amongst the children of the hill tribes, it was practically absent amongst the adult population of the hill tribes

Further the detailed study of the spleen census of children, age by age, showed a definite law regarding the splenic enlargement of children To explain this, I append herewith the curve which was published in my paper named 'A Peculiarity in the Spleen Rate as Observed in the District of Chittagong Hill Tracts,' which appeared in the *Indian Journal of Medical Research,* April 1921 In this curve the percentage of enlarged spleens for each year has been regarded as ordinate and respective years of age of children as abscissa The

curve, after rising somewhat up to the age of three years, shows a very regular and steady fall to the age of twelve, the fall from the seventh to the twelfth year being somewhat more abrupt than that from the third to the sixth year



It has subsequently come to my notice that Lieut-Col S P James, IMS, in his book 'Malaria at Home and Abroad' has published a curve which is very similar to that obtained by myself

Three and a half years later Christophers published his paper, 'The Mechanism of Immunity against Malaria in Communities Living under Hyper-endemic Conditions' in the *Indian Journal of Medical Research, October* 1924, which may be regarded as one of the most comprehensive pronouncements on the question of immunity in malaria, the subject I tried to study in my previous paper The area and the people in which and amongst whom Christophers carried on his investigation was very similar to the locality and the people concerned with my investigation In this present paper I shall utilize some of the results arrived at by Col S R Christophers

At the time of carrying out my malarial survey of Chittagong Hill Tracts during the years 1917 and 1918, the peculiar nature of the fever attracted my attention Attention to this was drawn by the following remarks in my paper in this *Journal* already referred to —

'The remittent or continuous type of fever which is frequent in the malarial districts of Bengal is not usual among the hill children In fact during my stay in the district, cases of continued fever noticed by me have occurred as a rule amongst the non-hill tribe population The very few cases of continued fever I have seen amongst the hill children have all occurred in very young children under two years of age, and some of these have been fatal' In the clinical notes on the few cases published in my paper it was stated that though all the cases are suffering from marked enlargement of spleen, several of the cases are afebrile, some cases showed a rise of temperature occasionally as for example 2 or 3 times a month Sometimes there was only a slight continuous rise of temperature 3 or 4 days at a time

After reading the researches of James, as well as those of Christophers previously referred to, I found myself again interested in the study of the malarial temperature charts in the Chittagong Hill Tracts I was able to secure some temperature charts from the district through the kindness of Dr L M Roy and his successor Dr R Ghosh, Civil Surgeons of the district But for their kind favour, I would not have had materials to write this paper I should mention here that Dr L M Roy was in very bad health when I asked him for the temperature charts and he ultimately died without recovering his health But his broken health did not prevent him taking trouble in the matter and he requested his successor to continue the help to me, when he was compelled to take leave on account of his illness

To elucidate some points in my present paper, I quote below a few extracts from the excellent paper by Christophers, on the subject of 'Immunity against Malaria under Hyper-endemic Conditions'

'Among the 18 children of age 1-2 resident on the estate for at least 12 months the average parasite value was 12,084, and the infections encountered ranged from 83,000 to 140 parasites per c mm Assuming that each

child had the same history as regards cycles of parasites as the others, we may, for the purpose of illustration, make the following approximate estimate of what an individual child passes through As 18 children examined at random at a given time had 5 infections of 10,000 per c mm, one child of these 18 is likely in 18 days, on an average, to have 5 such infections Similarly, for 13 out of the 18 days it ought to have over 1,000 parasites per c mm, and no day without parasites The significance of these findings is further appreciated when it is considered that, as a result of counts of parasites in *attacks* among seamen, Ross and Thomson (1910) fixed 200–500 in the case of *P vivax* and 600–1,500 in the case of *P falciparum* as the number of parasites per c mm required to cause fever From counts given by these authors it would seem that from 3,000–4,000 parasites per c mm were invariably associated with an attack, and a temperature rising to 103°F or over Taking even 5,000 parasites per c mm as a convenient test number for an infection sufficient to cause an attack, each of these children must be considered as under 'attack' conditions in respect to the number of parasites in their blood on 8 out of 18 days at least Not only so, but the number of parasites would represent attacks of some severity The highest count in the series is only once exceeded in Ross and Thomson's figures, and out of 30 attacks in the seamen, only 8 are as high as the next four higher in the children This degree of infection, also, was not during the fever season when one might suspect it to be temporary, but at a time when malaria transmission was probably least active

'There seems little doubt, therefore, that this intense infection, that can only be described as continuous attack, with an average parasite count of over 10,000 parasites per c mm, and lasting something like two years, is an essential feature of hyper-endemic conditions I shall call it the stage of acute infestation It is extraordinary to consider that a small baby should be able to pass successfully through a two-year-long attack of malignant tertian, which is what such a stage amounts to

'In children of age 2–5, who have been resident on the estate for at least three years, the proportion infected is still as high as ever, but the average value of the infections is now altogether different, for the average number of parasites is now only 1,200 per c mm, and the highest infection encountered one of 5,420 parasites per c mm For the sake of illustration we may say that at this stage the child now only has an attack about once in 25 days

'Through age period 6–10, among those who have passed through the ordeal of acute infestation, the numerical value infections was under 1,000 per c mm, though the actual infection rate was still approximately 100 per cent The period following acute infestation and lasting through childhood to adolescence we may call the stage of immune infestation

'The net result of childhood passed under hyper-endemic conditions is the acquirement of a form of immunity It is probable that immunization is produced not by infections and attacks scattered through the age of childhood,

as I think has generally been supposed, but by a period of terrific parasitic infestation lasting some two years, followed by a changed state in which parasites are still to be found but in small numbers The period of acute infestation should normally start shortly after birth, but in the case of immigrants may commence at a later age'

There is a fact mentioned in the above extract the importance of which appears not to be appreciated often at first sight This is what has been described as acute infestation by Christophers

After completing my spleen curve of the hill children of Chittagong Hill Tracts, I consulted Dr Khambatta, Assistant Director of Public Health, who was deputed then to the work of malarial research under the Public Health Department of Bengal, as to whether a curve, similar to one obtained by me, can be constructed out of the materials of splenic census of children taken by his department for any place in Bengal Dr Khambatta very kindly took time to ascertain this by actual trial with the statistical data available in his department and in the end gave his answer in the negative Now what may be the reason that Dr Khambatta could not get a curve of splenic census like that of the Chittagong Hill Tracts even in the hyper-endemic malarial area in the plains of Bengal? The researches of Christophers have demonstrated the reason, which is as follows In the hyper-endemic area of Singhbhoom as well as that of Chittagong Hill Tracts there is happening acute infestation in consequence of which immune infestation is uniformly occurring amongst the hill children But the hyper-endemic areas, which were under Dr Khambatta's observation, were probably post-epidemic hyper-endemic areas, at least these were areas where the phenomena of acute infestation as well as immune infestation were not present in a degree to produce the phenomenon of immunity almost universally as was observed amongst the children of the hill tribes

The curve can be explained by the facts of acute infestation and subsequent immune infestation as have been pointed out by Christophers The child soon after birth goes through the phenomenon of acute infestation, which causes a rise in the spleen curve up to about 3 years Subsequently there is immune manifestation, which causes a steady fall in the spleen curve

If we study the temperature charts of malarial cases of a place where immunity is taking place through the phenomena of acute infestation and immune infestation and compare these with temperature charts of a place, in the same district, which is not hyper-endemic and where there is no acute infestation and immune infestation we shall notice some differences

I took spleen censuses in different parts of Chittagong Hill Tracts and I found this to be lowest at the part of the district known as Mahalchari where the spleen rate was 38 per cent I give here some temperature charts of children of this part of the district As Mahalchari area is not hyper-endemic the splenic enlargement amongst children is not so noticeable here as in the other parts of the district

Plate XXVII, Chart 1 is the temperature chart of a male child named Khija aged 6 years without any splenic enlargement belonging to Magh caste of the hill tribes

Plate XXVII, Chart 2 is that of a boy named Khijan aged 11 years having splenic enlargement of two finger breadths belonging to the Magh caste of the hill tribes

These two temperature charts have been prepared by S A S Satish Chandra Khan, in charge of Mahalchari Dispensary

These temperature charts may be compared with those taken in two other parts of the district which are hyper-endemic areas The following are some of the temperature charts of children taken in some villages of Ramgarh Thanah, the splenic rate being about 80 per cent

Plate XXVII, Chart 3 is that of a male child named Chingthu aged 3 years belonging to the Magh caste of the hill tribes The spleen in this case is one inch below the umbilicus and the liver half an inch below the costal margin

Plate XXVII, Chart 4 is that of a female child named Ombai aged 6 years belonging to the Magh section of hill tribes The spleen is 2½ inches below the costal margin

These temperature charts have been sent by S A S Prasanna Kumar Barua, in-charge of Ramgarh Dispensary

The temperature charts which are given above taken during the non-malarial season have a resemblance to those of primary fever described by Col James But in the malarial season we get attacks like malarial fever distributed at irregular intervals The following are a few clinical histories which I collected when I was at Chittagong Hill Tracts with the result of blood examination These clinical histories have been published in my paper in the *Indian Journal of Medical Research*, April 1921, which are given below —

1 Foreya Chakma male, age 12, race Chakma A huge benign tertian parasite found Spleen just palpable The boy occasionally gets fever, but at the time of taking the blood film there was no fever His mother stated that the boy had suffered much from fever in his earlier years

2 Botal Bhusan Dewan male, age 12, race Chakma Malignant tertian parasite detected The boy is in poor health, suffering from scabies and other skin diseases Spleen extends three finger breadths below the costal margin The boy has not suffered seriously from fever During the last six months he has only had fever once, and that only for two or three days The boy had no fever when the film was taken

3 Ciyoshi female, age 3 years, race Magh Malignant tertian rings were detected by Dr Maitra She had an enlarged spleen three finger breadths below the costal arch She suffers from intermittent pyrexia of tertian type, i e, she gets fever at every third day for three or four days at a time Fever is preceded by chill and rigor Every attack of fever has a cold stage for about an hour and then a hot stage for four or five hours and the fever remits with sweating The duration of this ailment is said to be of more than a year The child suffers from this type of fever twice or thrice in a month

4 Radha Kishore male, age 4, race Tipperah Dr J C Mukerjee found malignant tertian rings The spleen is enlarged four finger breadths below the costal arch He suffers from intermittent fever of malarial type

Plate XXVII

Chart 1

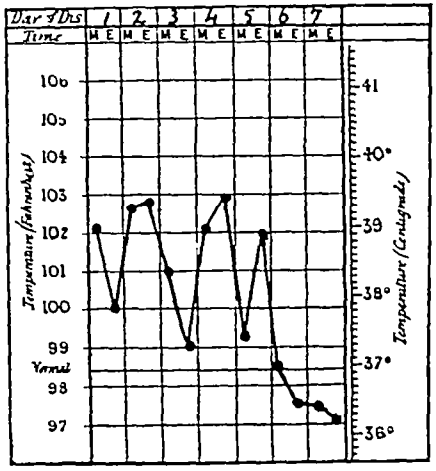


Chart 2

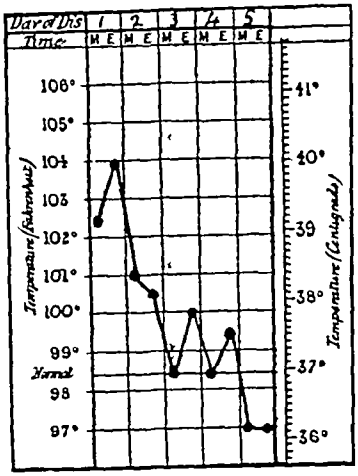


Chart 3

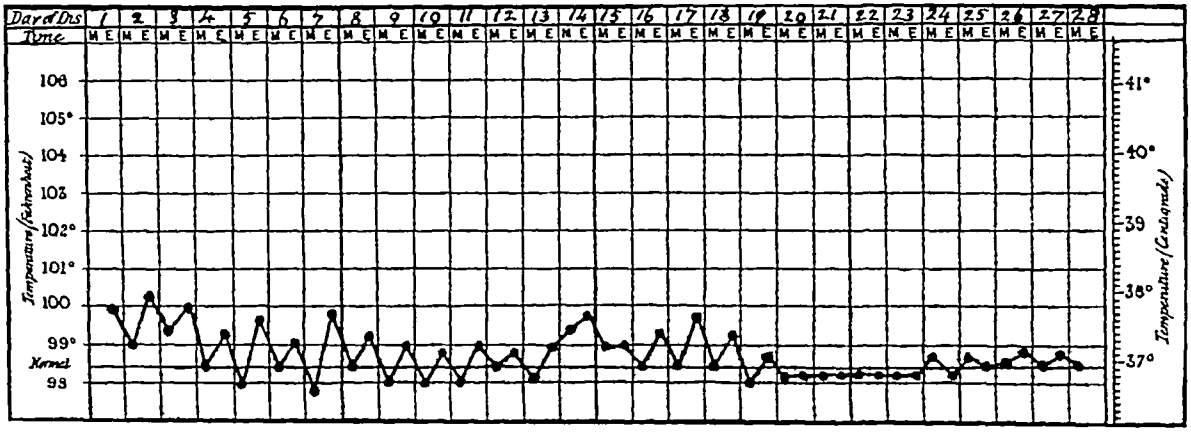


Chart 4

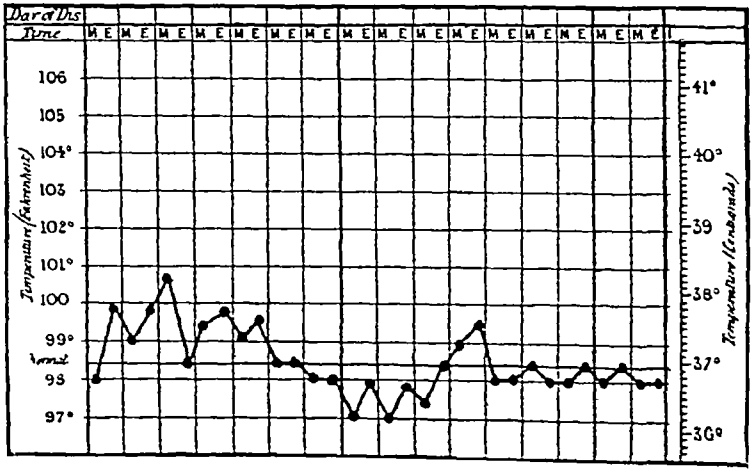


Chart 5

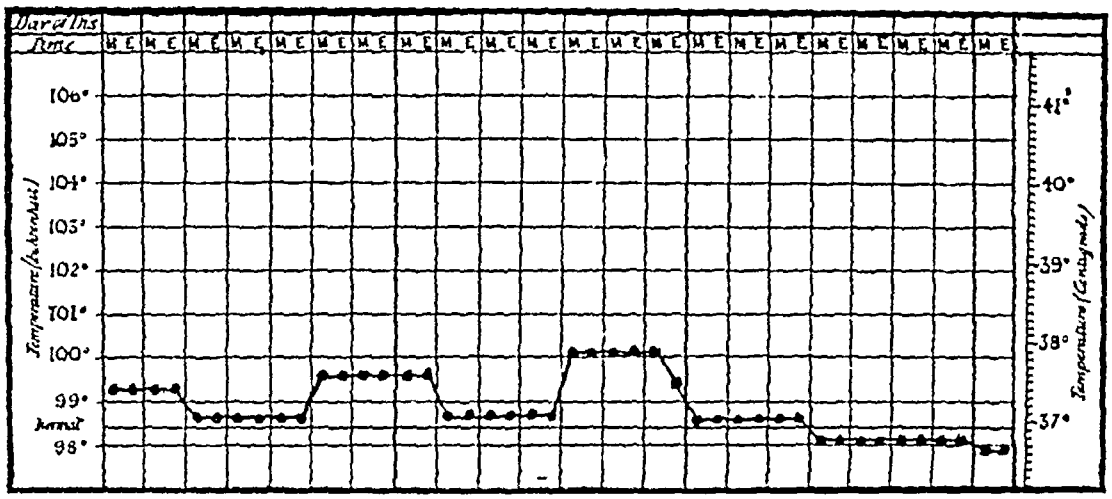


Chart 6

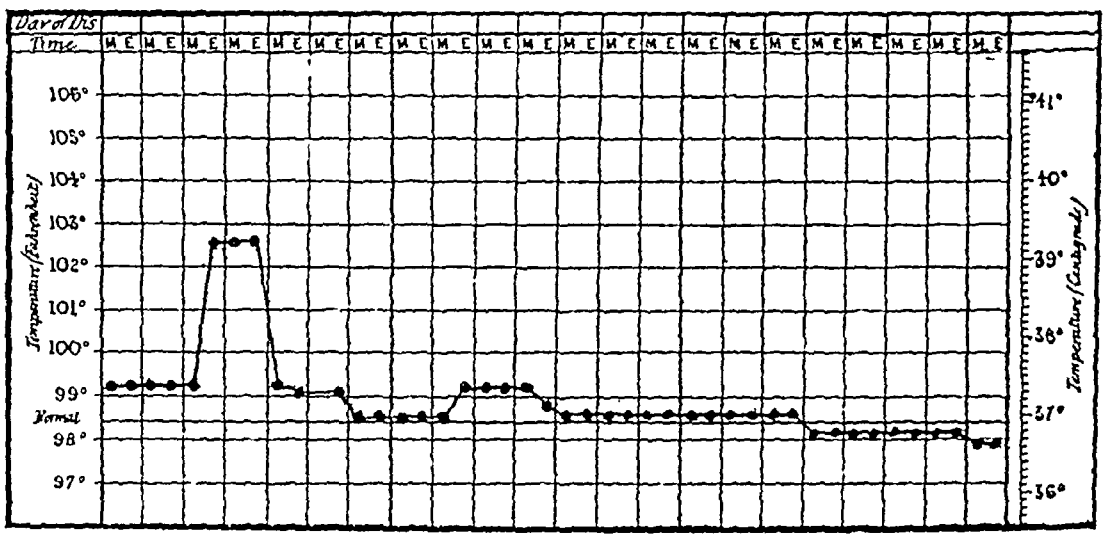
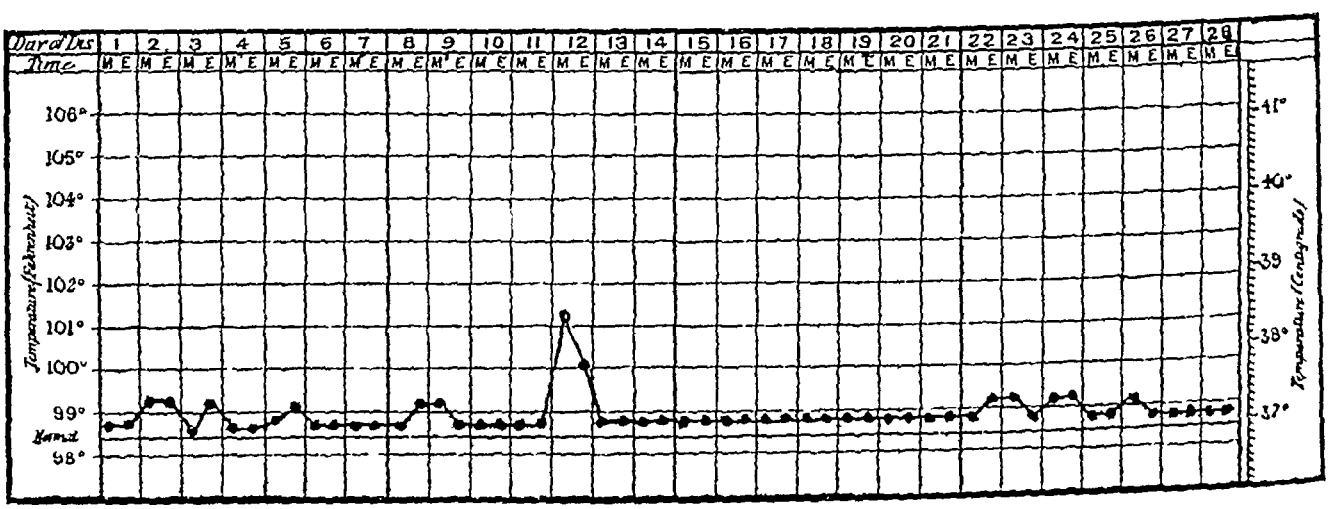


Chart 7



5 Gayaswari female, age 2½ years, race Tipperah Dr J N Maitra found innumerable rings of malignant tertian parasite She has an enlarged spleen three finger breadths below the costal arch She gets fever four or five times a month of malarial intermittent type

6 Panchaswari female, age 8 years, race Tipperah Dr Mukerjee found malignant tertian rings The spleen extended to the umbilicus She gets fever at intervals of one or two days Fever is preceded by chill and rigor Duration of the disease is said to be three years

7 Satin Kumar male, age 4 years, race Tipperah Dr Maitra detected innumerable malignant tertian rings in the blood The spleen extends three finger breadths below the costal arch He gets fever once or twice in a month Fever is of quotidian type and is preceded by chill and rigor The duration of the disease is given as about two years

8 Lakshmipaty female, age 2½ years, race Tipperah Dr Mukerjee detected malignant tertian parasites Spleen is four finger breadths below the costal arch She gets fever frequently Fever is preceded by chill and rigor and lasts with continuous rise of temperature three or four days at a time Her liver is enlarged

9 Aphoo female, age 4 years, race Magh Dr Mukerjee found malignant tertian parasites in the blood Spleen extends four finger breadths below the costal arch She gets fever every day, sometimes every third day Fever is preceded by chill and does not last for more than seven or eight hours She is a little anæmic Digestion not good Bowels irregular, often loose

In the above clinical notes the following points of interest may be noticed — The outbursts of malarial fever take place at irregular and as a rule after pretty long intervals Though all the children are infected with tertian parasites, the types of fever are different Case No 7 is of quotidian type In cases Nos 6 and 9 it is sometimes quotidian and sometimes tertian The mechanism of this can be understood by the light thrown by the researches of Col James In case No 8 it is stated that there is a continuous rise of temperature three or four days at a time A typical temperature chart of this kind can be seen in the chart of a Hindu Tipperah boy aged one year with splenic enlargement of 1½ inches below costal margin given below, with two other temperature charts of hill children The following are the clinical notes about the charts —

Plate XXVIII, Chart 5 Name, Bashanchan, caste, hill Tipperah (Hindu), age, 1 year Spleen enlarged 1½ inches below costal margin

Plate XXVIII, Chart 6 Name, Taralhari, caste, hill Tipperah (Hindu), age, 1½ years Spleen was palpable

Plate XXVIII, Chart 7 Name, Mounchawo, caste, hill tribe, age, 2 years Spleen 2 inches below the costal arch

These charts have been sent by Sub-Assistant Surgeon Abu Zaffar Mohamed Samsuddin of Dighinala Dispensary

Now if we compare these temperature charts with those of Mahalchari side which is not a hyper-endemic area we shall notice the following differences —

In Mahalchari side temperatures rise to 103°F and frequently above, but this is not frequent in the temperature charts of Dighinala side In the temperature charts of Mahalchari side, there is an abrupt rise and fall, but in

the temperature charts of Dighinala side, there is the tendency for the temperatures to remain at constant levels for some hours after rise, so that the apical points of the temperature charts of Mahalchari side present peaks, while the apical points of the temperature charts of Dighinala side resemble a series of flat plateau with level tops The temperature charts of Mahalchari area resemble the temperature charts of ordinary malarial fevers as met with in plains of Bengal, but the temperature charts of Dighinala area are not so because the outbursts of fever do not correspond with the regular periodicity of ordinary malarial fever Moreover, the rise of temperature is not abrupt and high as met with in ordinary malarial fever If the temperature charts are arranged according to age, it will be found that in the hyper-endemic area, i e, on Dighinala side, the acuteness of fever, as indicated by the height of the rise of temperature, shows a tendency to diminish with the advance of age, though there are exceptions But no such tendency is manifest in the sub-endemic area, as on Mahalchari side

Another point of difference between the malarial fevers of the hyper-endemic area and sub-endemic area is this Though in the hyper-endemic area the manifestation of fever is of sub-acute type as compared with that of fevers of sub-endemic areas, yet this low type of fever does not yield as readily to quinine treatment, as the more frank types of malarial fever in the sub-endemic area Some of the reasons for the same we can find in the researches of Christophers He has shown that the infection by crescents varied with the numerical value of parasitic infection (asexual) per c mm in the hyper-endemic area in which he examined the children From analogy we may conclude that the same state of things prevail amongst the children of Chittagong Hill Tracts, as there is evidence of similar acute and immune infestations of children in this region At Singhbhoom Christophers found crescents amongst children as high as 392 per c mm and the average value as 96 below one year's residence, 59 in children of one year's residence, 53 in children of two years' residence and 37 in children over two years' residence Such a high degree of crescent infection is perhaps sufficient to explain the relapses under a treatment with insufficient dose of quinine as shown in the temperature charts given below Besides this there may be other reasons as well The parasitic index in hyper-endemic area as has been shown in the paper of Christophers is pretty high, as compared with that in a sub-endemic area We would naturally expect that in fever cases where the parasitic index is high, children will be cured with more difficulty, will suffer more relapses, than in a case where the parasitic index is low

Lieut-Col S P James, I M S, in Byam and Archibald's 'Practice of Medicine in the Tropics,' Vol II, page 1634, has stated, 'In all probability quinine has little effect on parasites in red cells in the capillaries of internal organs and deeper tissues, Ramsden and Lipkin have adduced evidence bearing on the possibility that in the blood vascular system there are regions which are kept almost free from quinine throughout a period of quinine treatment'

Now if the parasitic index is high there is more chance of greater number of parasites escaping the action of quinine by staying in this region than when the parasitic index is low

I append herewith the temperature charts of children under the administration of quinine collected from Banderban side of the district where the splenic index is about 75 per cent

Before concluding my paper, I shall earnestly thank Dr L M Roy, Dr R Ghosh, the previous and the present Civil Surgeons of Chittagong Hill Tracts, Sub-Assistant Surgeon Prasanna Kumar Boruah of Ramgarh Dispensary, Sub-Assistant Surgeon Abu Zaffar Mohamed Samsuddin of Dighinala Dispensary, Sub-Assistant Surgeon Satish Chandra Khan of Mahalchari Dispensary and Sub-Assistant Surgeon A F Ali Hasan of Banderban Dispensary all of whose kind help and co-operation have made it possible for me to write the present paper

Conclusions

(1) Lieut-Col S R Christophers, I M S, has proved that hyper-endemy is a special form of endemy carrying its own particular conditions compounded of the functions of infection and immunity under a particular class of circumstances

(2) The temperature charts of malarial fevers under hyper-endemic conditions are somewhat different from ordinary malarial temperature charts

(3) The malarial fever cases of the hyper-endemic areas are more resistant to quinine treatment, and are more liable to relapses than ordinary malarial fevers

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MALARIA AND BLACKWATER FEVER AT NOAMUNDI

BY

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THROUGH the kindness of Mr Senior-White of the Bengal Nagpur Railway Malaria Prevention Service we were in August 1929 enabled to make a few observations on some of the conditions associated with blackwater fever and malaria at Noamundi, a railway settlement* in the Singhbhum District of Chota Nagpur and, as a control to these observations, some others in an adjoining valley, that of the Betlata, which is the home of the aboriginal Kols (*see* Map)

PHYSICAL FEATURES OF THE COUNTRY

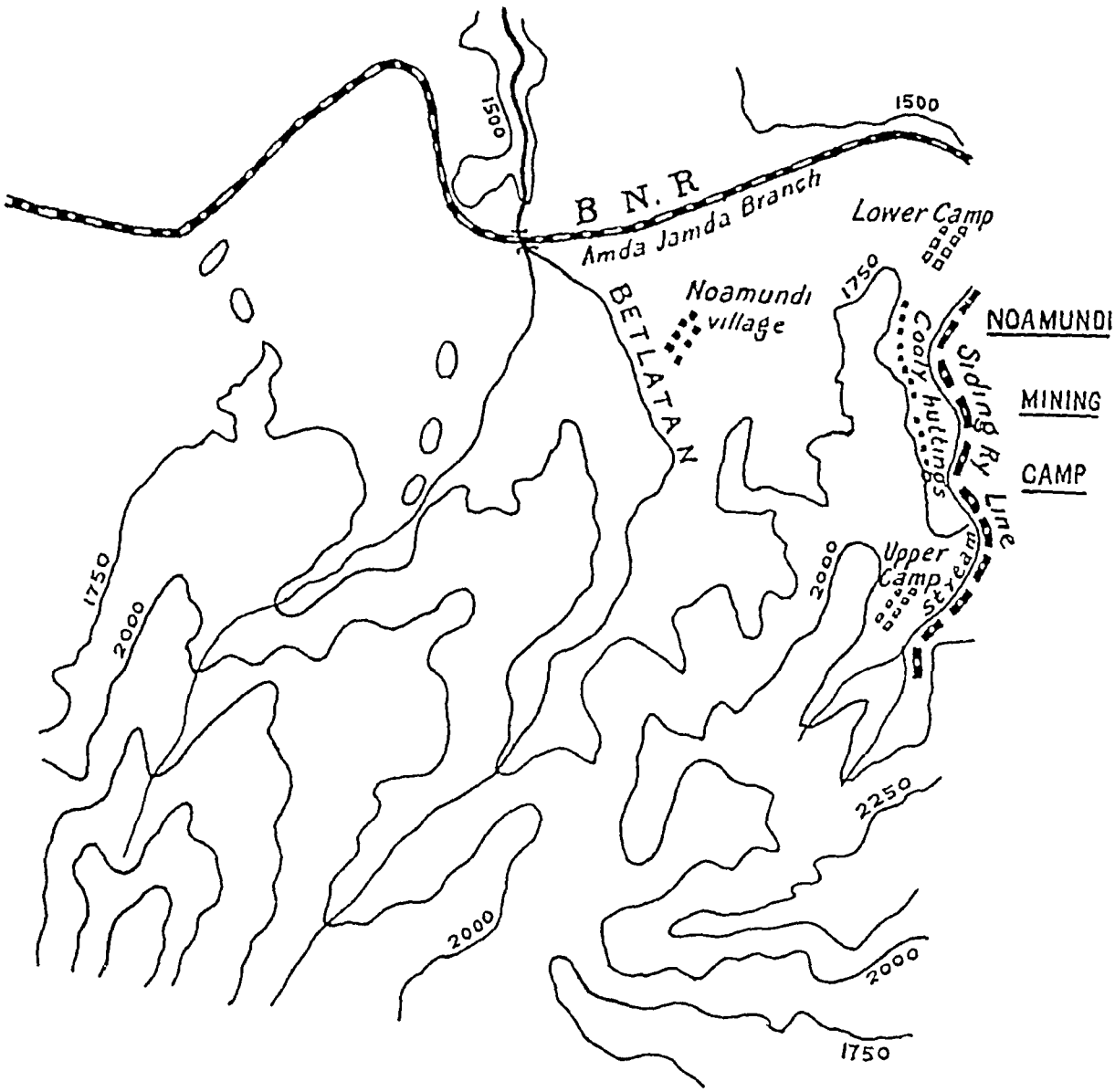
The country here is undulating with low hills and valleys In each valley there are one or more streams into which trickle hill-foot springs The hills and valleys are naturally covered by forest but are nude where mining is going on, and a few of the valleys outside of the mining concessions are cultivated Noamundi mining camp lies between 1,400–1,800 feet altitude in one of the little valleys, and this has approximately the following dimensions —

Length, north–south, nearly two miles
Breadth, east–west, a little over a mile

The soil is murram with exposures of hematite and patches of banded quartz and laterite

*About a mile to the south of the Bengal Nagpur Railway line (Amda-Gua Branch line) and a mile and a half from the Noamundi railway station with which it is connected by a broad gauge siding line Lat 22° 9 N Long 85° 29 E

MAP



THE CLIMATE

Average annual rainfall	64 inches (normal)
Wet months	3
Dry „	9

We give our observations of these two areas side by side when the data are available and for much of the information we are much indebted to Mr B B Mitra, the manager of the mining camp

SHORT HISTORY OF THESE SETTLEMENTS

There was a temporary settlement while the work was started in 1923, then in 1926 the camp was shifted to a new, the present, site, in permanent buildings

Water supply—Two natural springs are collected into a reservoir, 300 feet higher than the camp levels, to which there is a pipe-supply

Our survey

We now give the results of our survey in this part of the country and the conclusions to which we think they lead

Spleen-indices

Iron-mines camp

Knowles (1929) says that in March 1929 the S I x was for the aboriginals 58 per cent, and for Bengalees 40 per cent

We obtained the following figures —

	exd	*pos*	*S Ix*
Upper camp, Bengalees	5	2	
„ „ Ooriyas	16	4	25
Cooly-huttings—			
Nos 2 and 3, Ooriyas	50	24	48
Cooly-huttings—			
No 1, Ooriyas and Kols	43	30	70
Total	114	60	44

Blood parasitised-children-index and specific indices Iron-mines camp

	Films *exd*	Films *pos*	*P Ix*	*Specific indices* M T	B T	Q
Upper camp babus lines, Bengalee	5	2		50	50	
Upper camp cooly lines, Ooriyas	14	9		43	30	
Cooly-huttings—						
Nos 2 and 3, Ooriyas	21	8			24	14
Cooly-huttings—						
No 1, Ooriyas and Kols	16	9		15	23	
Total	66	28	43	17	26	5

Prevalence of blackwater fever and malaria

Iron-mines camp

A schedule is attached (Appendix I) showing the record of blackwater cases in the camp from December 1927 to January 1930 They amount to 21 including one imported

The only points to which we here draw attention are these —

	Race	*Occupation*
Racial incidence and occupation	Bengalees	17 ministerial establishment
	Punjabi	1 artisan
	Ooriyas	2 coolies
	Gurkha	1 cooly

As the coolv population here greatlv outnumbers the superior classes the very great predisposition to b w of the latter is as marked at Noamundi as in the Bengal Duars, or the Terai, as has been reported on elsewhere *

Thus taking the population in 1928 as the average we calculate that —

in	*there were cases of b w*	*i e, rate per mille about*
17 officers	0	
280 clerks and other ministerial establishment and artisans (excluding imported case)	17	61
1311 coolies	3	0·2

These figures cover, it must be remembered, a period of 26 months, and are comparable with a rate of 312 per mille in the Terai for a period of 11 years The respective coolv-rates were nevertheless about the same notwithstanding this difference of time, and would indicate that the coolies living under the conditions pertaining at Noamundi are more affected than a tea-garden population

There were records of quinine treatment in 10 of the 20 *non-imported* cases

The length of residence previous to attack varied from 5 months to 3 years

The month of attack (*vide* the records in Appendix I) was markedly in the non-monsoon season

There were 16 b w habitations in the higher camp and 3 in the lower

Malaria sickness at Noamundi non-mines camp

The incidence of malaria was definitelv vernal and autumnal

Some annual incidence records were as follows —

	Malaria cases	*Population*	*Rate per mille about*
1927	5,044	2,590	2,000
1928	8,268	1,679	5,000

The Bengalees, Punjabis and Beharis were the most susceptible to malaria

Punjabi mechanics at work on railway construction have had to be repatriated every 3 months owing to constant and severe malaria

With regard to local distribution, the lower parts of the camp were considered to be less malarious than the upper

Other diseases specially prevalent were pneumonia and dysentery

Mosquitoes

Adults—The following table shows the adult catch from 18th August to 15th September, 1929

* McCutcheon (1928), Strickland and Chowdhury (In Press)

Adult mosquitoes caught in Noamundi during period 18th August to 15th September, 1929

	culicifacies	*fuliginosus*	*funestus*	*karwari*	*maculatus*	*maculipalpis*	*philippinensis*	*rossi*	*theobaldi*	*vagus*	TOTALS
Male	87		2					41			130
Female	442	4	17	41	6	11	1	148	10	9	689
TOTAL	529	4	19	41	6	11	1	189	10	9	819

We draw attention to the small catch of *maculatus* (6) while *culicifacies* (529) was far more prevalent than *funestus* (19)

LARVÆ

We first oppose the record of the catch from the Noamundi mine area to that from the adjoining Betlata River valley and have calculated each as for a period of 100 days (the actual catches are shown in Appendix II)

	Betlata valley	*Non-mine area*
A aitkeni	65	
A barbirostris	18	
A culicifacies	1,842	11,546
A fuliginosus	1,030	555
A funestus	5,930	991
A hyrcanus	65	
A jamesii	277	646
A jeyporiensis	1,036	437
A karwari	53	210
A maculatus	2,977	3,900
A maculipalpis	683	919
A majidi	336	
A theobaldi	71	382
A vagus	165	10
A rossi-vagus		10
A rossi		7,255

The great increase in the mined valley of *culicifacies* with a nearly corresponding decrease of *funestus* and in both *maculatus* about equally prevalent, are the most noticeable features of this comparison

The types of breeding-place in the two valleys are indicated in the tables in Appendix II

'Standing water pools,' very productive of *culicifacies* and *subpictus* (grassi), were common in the mines area and due to the operations of the mines labour, whereas they were absent in the Betlata valley. There were also two other types of breeding-place in the former absent in the latter, viz., a swamp on the river-bank (of 'R. W. 12'), and the Kurta Dam tank, but neither of these were very productive of Anophelines.

Discussion

A malarial aetiology of b. w. is assumed, but the considerable prevalence of b. w. at Noamundi is rather marked in view of the spleen-index of about 14 per cent, which is not an 'hyper-endemic' rate. The malaria sickness rate in 1929 was about 5,000 per 1,000.

The correspondence in the seasonal-prevalence of b. w. and of malaria was very noticeable (the '*culicifacies type*,' not the '*funestus*'), and affords further proof that the b. w. syndrome, although on occasions much delayed, is usually not so.

With regard to racial incidence we found that the malaria-susceptible foreigner (Bengalees) suffer from b. w. much more than 'the native' in a strikingly similar degree (allowing for differences in the periods of the survey) to what McCutcheon found in the Duars and we have in the Terai. That is not, however, because the 'native' is malaria-immune, but probably as we have pointed out in connection with the Terai, because of his living in a fumigated dwelling.

It is a popular belief that the drink habits of the cooly saves him from b. w. 'by flushing out his liver' but we have collected no figures in relation to this possible factor.

The fact that malaria and b. w. were more prevalent in the upper parts of the valley, 16 b. w. houses as against 3 in the lower, while the man-made breeding-places of *culicifacies* were much more extensive in the upper parts, suggests a *culicifacies* connection with b. w. This is further supported by the correspondence between the seasonal incidence of b. w. and malaria which was of the *culicifacies* type.

We have already in connection with the Terai deduced that *funestus* as apposed to *maculatus* is the agent of the induction of b. w. At Noamundi in the non-mine area also, *funestus* doubtless take its share, but it is so relatively scarce that we believe *culicifacies* to be the main source of the trouble. We rule out *maculatus* on the ground that elsewhere it is demonstrably not a cause of b. w.

In the Terai we had to explain the distribution of b. w. on the ground of variations from an average infection-rate and this was only compatible with an hypothesis (which however was supported by figures) that *funestus* by its peculiar domestic habits footed the bill.

And as hypothetically any species of mosquito with such particular habits could be an agent for the high infection-rate necessary for the induction of

b w we believe that, in the non-mines area, *culicifacies* is such an agent Notoriously it is a 'domestic' species

We showed in our Terai report that the number of *funestus* adults to be caught in houses is compared with its larvæ caught in the neighbourhood was 16 times as great as the respective figures for *maculatus*

In the Noamundi camp area the following were the corresponding figures for the reputed pathophors

	adults	*larvæ*	*adults ratio larvæ about*
funestus	19	109	2/11
culicifacies	529	1,270	2/5
jeyporiensis	0	48	
all *myzomyias* as against *maculatus*	6	429	2/143

that is, in this area the 'domesticity' of *culicifacies* or of the *myzomyias* in general as apposed to *maculatus* was as 143/5 or about 30 to 1

Conclusion

The observations that we made in this Noamundi area lend support to the theory evolved by us from the data collected in the Darjeeling Terai that while blackwater is usually associated with hyper-endemic malaria, its irregular distribution, as exemplified by b w houses, is due to variations from an average malaria infection-rate leading to a more intense local infection-rate sufficient to induce the condition

A spleen-index of any size from 0 to 100 may hypothetically represent an average infection rate per person either sufficient or insufficient to induce b w As, however, a spleen-index of 100 per cent often does not lead to b w we must presume that unless the average infection rate is *n* times the minimal rate that can produce this spleen-index b w will not arise, but if the necessary rate be attained all the persons in a community will get b w As this never occurs we must conclude that an average rate applied to all members of a community never happens Variations from the average infection-rate of a community lead to more intense infection-rates in some and less intense in others and when the critical infection-rate is reached the b w prodrome is induced, so that ex-hypothesi if the local variation is sufficient a minimal spleen-index may be associated with b w, or if the variation from the average is not sufficient even a 100 per cent spleen-index may not be associated with b w It is only a matter of chance

In the concrete, at Noamundi what was not a so-called hyper-endemic malaria-rate, viz, 44 per cent, was associated with much b w

The necessary variation in the malaria infection-rate is, as we have concluded for the Terai, the function of the species *A funestus* Sufficient variation from an average infection-rate, in itself sufficient to yield a 100 per cent spleen-index, is not a function of *A maculatus* The reason for the difference doubtless lies in a difference in habits, one of which is a certain

APPENDIX I—*concld*

Serial No	Names	Age	Sex	Race	Occupation	Length of residence	Date of attack	Number of attacks	Quinine taken or not before attack	Cured or died	Remarks
11	Naibu	32	Male	Gurkha	Chowkidar	Five months	30-9-28	One	No	Cured	
12	Nalini Mukherji	26	„	Bengalee	Mines inspector	Three years	8-11-28	„	Yes	„	
13	Kalidas Chakrabarty	24	„	„	Mining assistant	„	10-11-28	„	No	„	
14	Amar Chand	34	„	Punjabi	Loco driver	More than two years	12-12-28	„	„	„	
15	Narendra Lal Ghose	30	„	Bengalee	P W I	„	15-12-28	„	„	„	
16	B K Ghose	38	„	„	Head clerk	„	25-11-28	„	Yes	„	
17	K K Dey	28	„	„	Electrical foreman	Fifteen months	4-12-28	„	„	„	
18	S C Ghose	30	„	„		More than two years	3-6-29	„	No	„	
19	Dinabandhu Ghose	30	„	„	Contractor	Few months	16-10-29	„	Yes	Died	
20	S K Bose	32	„	„	B N Ry employee		25-10-29	„		„	Came here for treatment on 25-10-29 and died after a few hours He came from Dongoposi Station
21	Antarjami	25	„	Oriya	Cooly	More than two years	8-1-30	„	Yes	„	

Appendix II

Table II-A

Larvæ found in valley to west of Noamundi mines area

From 29-8-29 to 17-9-29

Breeding-places	*aitkeni*	*barbirostris*	*culicifacies*	*fuliginosus*	*funestus*	*hyrcanus*	*jamesi*	*jeyporiensis*	*larvari*	*maculatus*	*multipalpis*	*nigeri*	*theobaldi*	*vagus*	Totals
1 Streams	5	3	63	150	814	5	34	109	5	213	41	57	7	5	1 511
2 Pools on stream beds			218		37		3	3	4	240	5		5	7	522
3 Pools on stream banks			12							12	1			7	32
4 Seepage water pools	6				41					34	6			7	94
5 Paddy fields			20	25	116	6	10	64		7	63			2	313
Total	11	3	313	175	1,008	11	47	176	9	506	116	57	12	28	2,472

NOAMUNDI IRON MINES CAMP AREA ONLY

TABLE II-B

Larvæ of Anophelines collected in August-September 1929

Breeding-places	culicifacies	fuliginosus	funestus	jamesi	peditaeniatus	karwari	maculatus	multipalpis	nigerrimus	subpictus	theobaldi	vagus	Totals
R W 36			2		5								7
R W 12	285	6	45	19	6	1	108		1	26	3		500
Pools on bed of R W 12	532	5	39	7	5		17	21		108	7	1	766
Swamp on bank of R W 12								35					35
Rice fields near B N R bridge	11	16	7	6			6	18					61
Kurta Dam tank	10												10
Standing water No 1										23			23
" 3	33									4			37
" 5	17												17
" 18	60									311			401
" 19	101									171			272
" 21	10									1			11
" 23										1			1
" 24	10												10
" 26	1												1
" 27	2									63			65
" 30	5									2			7
" 35										9			9
" 37	40	20											60
Seepage water No 6	42	14								42			98
" 14				13		5	20				1		39
" 28	110						8			3	1		122
" 32											9		9
" 34	1		23	26	32	17	210	27		1	20		387
TOTAL	1,270	61	109	71	48	23	429	101	1	798	42	1	2,954

NOAMUNDI IRON MINES CAMP AREA ONLY

Summary of Table II-B

Larvæ of Anophelines collected during August-September 1929

Breeding-places	*culicifacies*	*fuliginosus*	*funestus*	*jamesi*	*jeyporiensis*	*karwari*	*maculatus*	*maculipalpis*	*rossi-vagus*	*subpictus*	*theobaldi*	*vagus*	Totals
R W 36			2		5								7
R W 12	285	6	45	19	6	1	108		1	26	3		500
Pools on bed of R W 12	532	5	32	7	5		47	21		108	8	1	766
Swamp on bank of R W 12								35					35
Rice fields near B N R bridge	11	16	7	6			6	18					64
Kurta Dam tank	10												10
Standing water Nos 1 to 37*	279	20								618			917
Seepage water Nos 6 to 34*	153	14	23	39	32	22	268	27		46	31		655
Total	1,270	61	109	71	48	23	429	101	1	798	42	1	2,954

*S W and S P Nos are not consecutive

OBSERVATIONS ON RAT-FLEAS AND THE TRANSMISSION OF PLAGUE

Part III

BY

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FLEA BIONOMICS

Man as a host for the Indian rodent Xenopsylla

OBSERVATIONS by the Indian Plague Commission (1907), Chick and Martin (1911), Bacot (1914), and others give ample evidence that *X cheopis* readily feeds on man The identity of the fleas used in some of the experiments is, however, open to doubt As regards *X astia*, Hirst (1913) reported that the species feeds on man with great reluctance at temperatures of over 80°F in Colombo, and Clagg (1923) came to a similar conclusion in Agra Taylor and Chitre (1923) found that *astia* would bite man readily in the Bombay cold weather The evidence regarding *X brasiliensis* appears to be confined to that of Ingram (1927), who reported that in S Africa this species is reluctant to feed on man

Hirst considers that fleas bred from the egg should be used in comparative biting experiments, but that exact comparison of the biting powers of different species of fleas is not possible, as some of the factors involved cannot be taken under control The writer has previously shown that individuals of the three species may exist for a prolonged period on an exclusive diet of human blood Since then, one male *astia* has been kept alive for 25 days under similar conditions

As a preliminary to study of the blocked-flea phenomenon a number of laboratory-bred fleas have been fed at intervals of not less than 24 hours on rats, guinea-pigs and a human subject respectively These fleas were all kept in test-tubes at laboratory temperature They were considered to have declined to feed if they had not bitten within two minutes of application The results are shown in Table I It should be noted that the fleas did not accept daily

TABLE I

Fleas offered daily feeds on different hosts

Fleas		Feeds on rat		Feeds on guinea-pig		Feeds on man	
Species	Sex	Offered	Per cent accepted	Offered	Per cent accepted	Offered	Per cent accepted
Cheopis	F	201	51	96	53	372	32
„	M	224	67	28	75	169	16
Astia	F	188	61	74	55	119	38
„	M	172	81	22	91	122	52
Brasiliensis	F	214	17			335	42
„	M	207	72			102	44

feeds even on the rodent hosts. In all cases males fed more frequently than females.

In the presence of a rat the fleas are not readily attracted to the human skin. For example, three transmission cages were prepared containing one rat and 40 fleas of the three species respectively. A human hand was laid on the floor of each outer compartment for five minutes, on two successive days, but no bites were recorded and no fleas appeared to reach the human skin.

A few examples of the daily behaviour of individual fleas fed on human blood are shown in Table II, which also indicates the temperature at which these observations were carried out.

In handling the breeding jars it has been obvious that hungry unfed fleas readily reach the human skin and begin to feed. This is much less marked in the case of *brasiliensis*, but this may be merely an indication of the lesser general activity of the species. It is noticeable that *cheopis* and *astia* are more active on the surface of the litter than *brasiliensis*, which, however, can be stimulated to activity by slight disturbance of the jar. Once disturbed they behave like the others. The high jump of all these fleas is no more than four inches.

In another experiment, laboratory-bred fleas were kept in cages with rats for six days to enable them to be regarded as mature fleas. They were then removed from the hosts and put in jars with litter. A dark-skinned Indian assistant, on the two following days, laid a hand on the surface of the litter in each jar for periods of five minutes, and the bites were noted. The surviving fleas were checked at the end of the experiment (Table III).

From these various results, it seems reasonable to conclude that starved individuals of both sexes of the three species feed quite readily on human blood. It does not appear that, other things being equal, the rat-to-man transmission

of plague in India would depend on the lesser attractiveness of man to one or other of these species

TABLE II

(a) *Fleas kept in pairs and applied to human forearm daily from day of emergence* (7-5-29)

Species	Sex	Days 1	2	3	4	5	6	7	8	9	10
Cheopis	F	—	—	+	—	+	—	—	+	—	+
„	M	—	—	D							
„	F	—	—	+	D						
„	M	—	—	+	+	—	+	+	—	—	+
Astia	F	—	+	+	+	D					
„	M	—	—	—	D						
„	F	—	+	—	D						
„	M	—	—	—	+	+	—	D			
T °F	D B	88	88	88	88	87	87	87	87	87	87
	W B	80	80	81	80	77	77	79	79	79	79

+ = Feed accepted, — = Feed refused, D = Dead

(b) *Fleas kept individually and applied to human forearm daily from day of emergence* (16-5-29)

Species	Sex	Days 1	2	3	4	5	6	7	8	9	10
Cheopis	F	—	+	—	+	0	—	—	+	—	—
„	M	—	—	—	D						
Astia	F	—	+	—	+	0	+	+	+	—	—
,	M	—	+	—	+	0	D				
Brasiliensis	F	—	—	—	+	0	+	+	—	+	—
„	M	—	+	—	+	0	+	+	D		
T °F	D B	87	87	86	88	88	87	87	87	87	87
	W B	79	78	79	80	77	78	79	79	80	78

+ = Feed accepted, — = Feed refused, 0 = Not fed, D = Dead

TABLE III

Mature fleas starved in litter. Bites on hand or forearm of dark skinned Indian (1 R)

	Number of	*Cheopis*		*Astia*		*Brasiliensis*	
		F	M	F	M	F	M
After 24 hours	Survivors	8	8	19	20	15	16
T. D. B. 75 W. B. 66	Bites	1	1	10	5	1	1
After 48 hours	Survivors	8	7	17	18	13	1
T. D. B. 77·5 W. B. 68	Bites	6	5	7	5	4	1

With regard to the subjective sensations, it is a general rule that the human subject is unaware of the bite of these fleas. Occasionally, and this has happened with both sexes of the three species, a tiny prick is experienced at the moment of biting. Immediately afterwards the site of the bite shows a red macule about a millimetre in diameter and this enlarges to about four times the size in the course of a few minutes. It then gradually disappears within a few hours. After a week, during which several dozen fleas had been fed to the same area of skin, there developed a papular eruption accompanied by some tingling and itching. The bite of *Pulex irritans* on the same subject has a similar result. There has been nothing resembling the 'acute pain of biting' described by Mitzmain in connection with the squirrel flea, *Ceratophyllus acutus* (1910).

The *cheopis* fleas fed on human blood laid many eggs, none of which hatched. One specimen laid eggs 68 days after it was isolated. No eggs have been noticed in the case of *astia* on a sole diet of human blood. One remarkable *brasiliensis* experiment suggests that this species finds man a suitable host. A wild female, isolated from males on November 5th and fed daily on human blood, laid at least 18 eggs in January and 13 in February, several of which hatched. The last to hatch was laid 114 days after the flea could have been fertilised.

LONGEVITY OF STARVED FLEAS

A large number of plague infected fleas being available, some observations on their length of life when separated from the host have been carried out. The results are shown in Table IV. The April conditions were hot and dry, while in June the monsoon had begun, the saturation deficiency for the month being only 0·211. The averages are calculated from the day on which the fleas were last seen alive, so that the true periods of survival are a little longer

TABLE IV

Comparative observations on the longevity of starved fleas from epizootic pits

Date	Conditions	Temperature and humidity	Fleas observed			Days	
			Species	Sex	No	Last survivor seen alive	Average survival
22-4-29	Individuals in test tubes	Av T °F D B 86 W B 80	*Cheopis* „ *Astia* „	F M F M	40 30 35 33	3 2 2 2	1·1 1·0 0·7 0·5
14-6-29	Do	Av T °F Max 85·5 Min 80·1	*Cheopis* „ *Astia* „	F M F M	10 10 10 10	6 4 7 5	3·3 1·4 2·4 2·7
15-4-29	Groups of 4 or 5 in test tubes	Av T °F D B 85·8 W B 78·6	*Cheopis* „ *Astia* „	F M F M	63 37 54 45	5 3 5 3	1·9 0·7 1·4 1·0
17-6-29	Do	Av T °F Max 86·0 Min 80·9	*Cheopis* „ *Astia* „	F M F M	41 25 54 49	5 3 3 3	2·2 0·6 0·8 0·7
29-4-29	Each group in lampglass on plaster of Paris base	Av T °F D B 87·0 W B 81·4	*Cheopis* „ *Astia* „	F M F M	50 50 50 50	7 4 7 7	3·1 2·0 3·9 3·2
19-6-29	Do	Av T °F Max 84·9 Min 80·7	*Cheopis* „ *Astia* „	F M F M	59 42 50 40	5 6 5 7	2·0 1·8 1·7 1·9
16-7-29	Do	Av T °F 81·6 S D 1·90	*Cheopis* „ *Astia* „	F M F M	48 47 45 44	6 7 7 6	3·0 2·7 2·4 1·8

than those shown Despite the considerable errors involved in these observations, it seems justifiable to conclude that, under natural conditions in the Bombay hot weather, individual starved fleas, both *astia* and *cheopis*, might live as long as a week, although the majority would die off between two and four days The best figure for *astia* showed 66 per cent of 50 females still alive after 96 hours starvation (Av T 9 A M 87°F)

Laboratory-bred fleas, collected on the day of emergence, have also been stored under different conditions, without having been given the opportunity of obtaining a blood meal, and the survivors have been checked daily These observations are recorded in Table V Individuals survived for two to three weeks at a temperature of over 80°F The survival of a few fleas for as long as three or four days in the dry incubator at blood-heat is a remarkable fact

TABLE V

Comparative observations on the longevity of unfed laboratory-bred fleas collected on the day of emergence

Period	Conditions	Temperature and humidity	Fleas observed			Days	
			Species	Sex	No	Last survivor seen alive	Average survival
Oct.-Nov. 1928	Lamp-glass on plaster of Paris base	Mean T 81°F S. D. 3·0	*Cheopis*	F	108	21	8
			"	M	20	17	9
			Astia	F	24	16	9
			"	M	28	17	10
			Brasiliensis	F	13	16	5
			"	M	56	20	7
Sept.-Dec. 1928	Lamp-glass in ice box	Mean T 68°F damp	*Cheopis*	F	98	28	8
			"	M	99	16	6
			Astia	F	22	10	7
			"	M	31	16	9
			Brasiliensis	F	16	15	6
			"	M	21	21	9
"	Lamp-glass in incubator	Mean T 98·1°F dry	*Cheopis*	F	76	1	1·5
			"	M	60	1	2
			Astia	F	16	3	2
			"	M	21	3	1
			Brasiliensis	F	18	3	1—
			"	M	28	0	1—
May 1929	Test tubes individually Lab. T	Mean T 87·9°F S. D. 1·05	*Cheopis*	F	16	9	5
			"	M	13	8	4
			Astia	F	11	12	7
			"	M	5	8	7
			Brasiliensis	F	8	9	7
			"	M	8	7	5

The majority of the experiments with infected fleas indicate that females outlive the males, while with new-born fleas, males in several cases had a longer period of survival. This may be accounted for by the difference in size of new-born and mature females, the former being much the same size as the males (*see* Table VI).

Fleas kept in the refrigerator at a temperature of 40°F. and in a saturated atmosphere were always apparently dead after 24 hours. It was found, however, that they would revive in a minute or two at room temperature and could be 'refrozen' and 'rethawed' for several successive days. The only comparative experiment under these conditions suggested that *astia* would be more rapidly killed off by extreme cold than *cheopis*. Fifty females and fifty males of each species from the plague-infected pits were kept in the refrigerator

TABLE VI

Length of fleas measured in capillary tube Average of twelve specimens in mm

Species	Sex	New-born	Wild
Cheopis	F	1·8	2·4
„	M	1·8	1·7
Astia	F	1·6	2·1
„	M	1·7	1·8
Brasiliensis	F	1·7	2·0
„	M	1·7	1·6

for 72 hours and the following revived at room temperature —*cheopis* 24 female, 17 male, *astia* 13 female, 5 male

It has been frequently noted that a moribund flea may succeed in getting a blood meal and survive

These figures cannot be readily compared with those of previous reports in which, as a rule, the sex of the fleas was not taken into account Some earlier observations are now known to have included a mixture of species Bacot (1914) reported that *cheopis* might survive 38 days unfed at a temperature of between 40° and 50°F The present figures do not demonstrate any marked or regular difference between the species in the powers of survival of starved fleas Wild or mature *brasiliensis* have not yet been studied There is evidence that the average life of starved fleas is a little shorter for *astia* than in the case of *cheopis*, but when individual survivors are considered, the difference is not marked It does not appear, therefore, that the period of survival of the starved flea can be an all-important factor causing a difference in the value of the different species as transmitters of plague, under Bombay conditions

NOTES ON FLEA BREEDING

In the course of handling a large number of wild fleas, and also mature laboratory-bred fleas from the epizootic pits, a certain number of eggs, laid by identified females, have been available from day to day With these have been included a number of eggs, chiefly *brasiliensis*, collected from time to time from a black cotton cloth spread on the floor of a breeding cage overnight Eggs laid on glass are allowed to remain in the same tube, as removal entails damage of a certain proportion From the cloth, the eggs are easily removed by a soft brush and stored in tubes or small Petri dishes The routine production of fairly large families of fleas in pure species for experimental purposes has incidentally provided some information on the reproductive powers of these species

Egg laying—As the majority of the fleas from which eggs were obtained were used for other purposes within a few hours, no detailed comparative figures will be given for egg laying in test-tubes The small number studied confirm the general impression that *astia* is a better egg layer under test-tube conditions than *cheopis*, and that *brasiliensis* is the least productive of the three There is very marked individual variation The *cheopis* and *astia* average in twenty-four hours is rarely over one egg per female, and the *brasiliensis* figure is usually less than 0 5 As will be shown later, such figures are much less than may obtain under more natural conditions

Egg hatching—The eggs were stored in the dark and examined daily, the appearance of larvæ being noted When the first larvæ appeared, a piece of dried blood-soaked rag was added to the tube, and when no further larvæ were expected, the tube was filled to a depth of half an inch with the usual sweepings The figures shown in Table VII are considered to give a fairly accurate idea of the fertility of the eggs, and the period elapsing between egg laying and larval emergence It is noticeable that in eleven months a higher percentage of larvæ was obtained from *astia* eggs than from *cheopis* The limited figures for *brasiliensis* indicate a very large proportion of productive eggs, the average being much higher than for the other two species The colder months of December and January show a larger proportion of productive eggs for both *cheopis* and *astia* The fall-off during February was probably due to the use of pulicides near the cup-board The delay in hatching in the colder months is clearly shown It must be noted that the day on which the eggs were collected is not included, so that a larva counted on the fourth day took from 72 to 96 hours to appear Some records probably count the day on which the eggs were laid as the first day The incubation period of *astia* eggs appears to be distinctly less than that of *cheopis* in most months of the year, while *brasiliensis* eggs on the whole hatch earlier than either

The only previous data of this type for Bombay are those of the Indian Plague Commission (1908) Working with a mixture of species, they found that flea eggs generally hatch in about two days, and they obtained 7 2 per cent larvæ from 461 eggs at room temperature (75°–80°F) Cragg's highest figure for *astia* in his Agra (1923) observations was 25 per cent larvæ from 262 eggs (T 86 4°F, relative humidity 69) In Colombo, Hirst (1927) obtained results similar to those now reported, the highest *astia* fertility being indicated by 71 6 per cent larvæ from 234 eggs (Av T, 80 8°F, S D, 0 23), the majority of the larvæ appearing on the fourth day His small number of *cheopis* eggs gave 40 per cent larvæ

Larvæ—The larvæ obtained as above have been followed to the imago stage It is considered that undue drying of the litter, when used in such small quantities, has interfered considerably with the later stages of development The necessary handling of the tubes is also liable to disturb the larvæ in the act of spinning the cocoon Adults have, however, been obtained under

these conditions in most months, and details are included in Table VII The duration of the various instars has been readily determined by observation of larvæ fed on blood-soaked rag only Thus in March, the first moult occurred in four to seven days, most often on the fourth day The second moult occurred

TABLE VII

Experimental flea breeding, Bombay, 1929-30

Month	Temperature and humidity	Species	Number of eggs observed	Days on which hatched							
				2	3	4	5	6	7	8	9
March	Av T °F 9 a.m D B 82 W B 75	*Cheopis* *Astia*	180 215		6	19 58	12 3	1 1	1		
April	Av T °F 9 a.m D B 85 W B 78	*Cheopis* *Astia*	495 353		28	119 137	15 8	2			
May	Mean T S D 87·9 4·05	*Cheopis* *Astia*	204 355		49	54 96	15 12	1			
June	82·9 2·11	*Cheopis* *Astia*	87 305		4 34	16 18	3 11				
July	81·6 1·90	*Cheopis* *Astia*	395 209		1	63 73	15 8				
August	81·8 2·19	*Cheopis* *Astia*	170 193			44 97	17 6				
Sept	82·6 2·40	*Cheopis* *Astia*	176 250		2	42 90	10 4				
Oct	82·6 2·85	*Cheopis* *Astia*	99 261			30 128	8 1				
Nov	80·3 3·20	*Cheopis* *Astia*	73 225			15 104	13 4				
Dec	74·8 2·72	*Cheopis* *Astia*	109 178			43	10 30	14 19	16 10	6	2
Jan	72·7 2·69	*Cheopis* *Astia*	93 42			2	19 6	17 8	15 13	4 1	
Feb	73·3 3·26	*Cheopis* *Astia*	139 137				10 8	11 7	9 11	1	
June	As above	*Brasiliensis*	81	4	36	14					
August	"	"	196	3	17	73	34	4			
Oct		"	118	1	40	36	1				
Nov	"	"	42			35	3				
Jan	"	"	234		2	46	119	17	6		
Feb ..	"	"	36				1	12	4		

about a week later, and cocoons were often formed on the 18th to 20th day The duration of the instars appears to be much the same in the three species Individual variation is a marked feature so much so, that active larvæ have been seen while newly emerged imagoes were being collected from the same batch

TABLE VII—*concld*

Month	Species	Larvæ		Adults			Days	
		Average time to hatch	As per cent of eggs	No	As per cent of eggs	As per cent of larvæ	Average period egg to adult	
							Female	Male
March	*Cheopis*	1 5	18 3	6	3 3	18 2	36	31
	Astia	1 0	31 6	17	7 9	25 0	28	30
April	*Cheopis*	1 1	27 5	6	1 2	1 1	21	33
	Astia	3 9	19 0	27	7 6	15 6	23	31
May	*Cheopis*	1 2	31 3	18	8 8	25 7	28	31
	Astia	3 8	11 2	31	9 6	21 7	29	32
June	*Cheopis*	1 0	26 1	2	2 3	8 7	29	35
	Astia	3 8	30 5	23	7 5	21 7	31	35
July	*Cheopis*	1 2	19 7	39	9 9	50 0	25	27
	Astia	1 1	39 2	31	11 8	37 8	25	30
August	*Cheopis*	1 3	35 9	35	20 6	57 1	29	32
	Astia	1 0	53 1	26	13 5	25 2	33	33
Sept	*Cheopis*	1 1	30 7	21	11 9	38 9	31	33
	Astia	1 0	37 5	35	11 0	37 2	32	35
Oct	*Cheopis*	1 2	38 1	12	1 1	31 6	32	34
	Astia	1 0	19 1	12	1 6	9 3	31	37
Nov	*Cheopis*	1 5	38 1	1	5 5	1 3	44	38
	Astia	1 0	18 0	6	2 7	5 6	32	35
Dec	*Cheopis*	6 5	11 0	15	13 8	31 3	19	56
	Astia	5 0	57 3	8	4 5	7 8	58	66
Jan	*Cheopis*	6 1	39 1	Nil	—	—	—	—
	Astia	6 2	71 1	Nil	—	—	—	—
Feb	*Cheopis*	6 0	22 3	Nil	—	—	—	—
	Astia	6 1	19 0	Nil	—	—	—	—
June	*Brasiliensis*	3 2	66 7	Nil				
August		3 9	82 1	70	35 7	13 5	30	33
Oct	,,	3 5	66 1	4	3 4	5 1	36	38
Nov	,,	4 1	90 5	Nil	—	—	—	—
Jan	,,	4 9	81 2	1	—	—	49	—
Feb	,,	6 2	47 2	Nil	—	—	—	—

Cocoons—Although cocoons were often formed on the glass and were sometimes so transparent that the contents could be observed, it is not possible to give figures for the proportion of cocoons resulting from the larvæ In the case of *brasiliensis*, in particular, the cocoons were often found to be covered over with fragments of rubbish, the little mass being unrecognizable as a cocoon without dissection The duration of the various stages inside the cocoon is of great interest but difficult to determine The folded larva has often been seen inside the pupa case for from 24 to 72 hours before the pupa was formed, and occasionally for as long as six days Fully formed imagoes have been noted for as long as seven days before they finally emerged The total duration of the cocoon stage was often about ten days in April, but varied from 7 to 16 days

Naked pupæ, both *astia* and *cheopis*, have been met with, and both female and male imagoes have been obtained from these at different times Bacot (1914) suggested that specimens of dwarf fleas might have had one larval instar less than normal It was noted that these naked pupæ might result in dwarf fleas, thus two male *astia* measured only 1 1 and 1 2 mm respectively (*see* Table VI)

Adults—The total development of the flea from egg to imago has varied from 19 to 66 days, under these conditions There does not appear to be any marked difference between the species in this respect Taking the figures for August, where there are adequate numbers for comparison, the averages are all within a day or two

The details indicating imagoes as percentages of eggs and larvæ respectively are not claimed as accurate, but the general trend of the figures may be indicated In the hotter months, a higher proportion of imagoes resulted from *astia* eggs than from *cheopis* eggs, while in the cold season the positions are reversed Similarly a higher proportion of imagoes developed from *astia* larvæ in March, April and June than from *cheopis*, while from October onwards the figure for *astia* is lower It is concluded that the colder weather in Bombay is less favourable for *astia* development

The complete failure of adults to appear in the January and February batches was disappointing, and obviously due to some error in the technique as noted in connection with the egg hatching

As regards flea breeding on a larger scale, some of the breeding jars were selected for accurate record of the output of young imagoes The jars were stored in a dark room and examined daily, newly emerged fleas being removed with as little disturbance of the litter as possible The results of these experiments, two of which, one in the hottest season and one in the cold weather, are strictly comparable, are shown in Table VIII The figures confirm the impression gained from handling a regular succession of breeding jars, viz, that *astia* was scarce in the cold weather and *brasiliensis* in the hot season, while *cheopis* was abundant in all months The temperature and humidity in these experiments may be judged from the details given in the previous

Table VIII

Comparative experiments in flea breeding, Bombay, 1929

No	Date of start	Species	Fleas used Number of F and source	Litter stored after days	Family recovered			Days from commencement until recovery of			
								First		Last	
					F	M	Av per female parent	F	M	F	M
10	5-10-28	*Cheopis*	11 wild	11	113	76	17	21	26	16	15
5	10-9-28	*Astia*	12 „	10	101	111	18	21	21	49	19
7	15-9-28	*Brasiliensis*	2 „	12	23	27	25	26	29	37	15
61	12-4-29	*Cheopis*	20 L.B	10	332	350	31	21	25	41	16
62	12-4-29	*Astia*	20 „	10	113	112	11	23	25	37	46
60	12-4-29	*Brasiliensis*	20 „	10	69	41	6	25	31	39	13
132	6-12-29	*Cheopis*	20 „	10	182	188	18·5	37	10	92	91
131	6-12-29	*Astia*	20 „	10	49	40	4·5	41	16	83	84
130	6-12-29	*Brasiliensis*	20 „	10	196	276	23·5	31	38	86	88

The number of male parent fleas used was identical with the number of females, except in Experiment 7 where no males were available

table, but it should be noted that the figures refer to the month in which the eggs were laid, while the further development of the fleas might extend into the following month and even into a third month

The remarkable delay in development with a fall in temperature of only 10° to 15°F is clearly shown The delay affects all stages Females have invariably been obtained for a few days before the first males appeared The proportion of females and males has shown no constant variation, and the numbers of the two sexes are often very close In the case of wild fleas, the results of surveys generally show a marked difference in the number of the sexes The most fertile jar gave an average of 34 *cheopis* per female parent as a result of ten days breeding As new-born fleas do not lay fertile eggs in the first 48 hours, the number of eggs per female must have been over four per diem even if no casualties occurred The laboratory stock of *brasiliensis* originated with two wild females, and it is interesting to note that in the absence of further fertilization, they produced fifty offspring

EFFECT OF EXTREME CONDITIONS OF TEMPERATURE ON THE DEVELOPING STAGES OF THE RAT-FLEAS

Eggs—No eggs have hatched either in the dry incubator at 98 4°F, or in the damp refrigerator at 40°F In a damp ice-box at a temperature of approximately 68°F there was a marked delay in the incubation period of the eggs in all species, and a fall-off in the output of larvæ in the case of *astia* (Table IX)

TABLE IX

Flea eggs laid at Lab T and kept at 68°F in damp ice-box

Species	Number observed	Av days to hatch	Per cent hatched
Cheopis	34	7 8	47
Astia	52	7 3	29
Brasiliensis	5	6·0	60

Larvæ—The larvæ may survive a brief exposure to highly unsuitable conditions Thus first and second stage larvæ, both *astia* and *cheopis*, have been kept in the incubator at 98 4°F, and also in the refrigerator at 40°F, for periods of 24 hours, along with the usual fodder, and adults have eventually been secured from all batches This exposure was, however, attended by a high mortality

Cocoons—*Cheopis* and *astia* cocoons, formed at room temperature, have been placed in the incubator at 98 4°F and observed daily Imagoes have appeared for the first three days but not later, and the unproductive cocoons have been found to contain dried up fleas or pupæ Exposure to this

temperature for only 24 hours did not affect the sub-sequent development at room temperature A *temperature of 10°F for a period of 24 hours was found* to have no harmful effect on the cocoons A marked fall-off in the output of adults followed exposure of the cocoons to this temperature for 48 hours (Table X)

TABLE X

Cocoons formed at Lab T and kept temporarily at 10°F (damp)

Species	Number observed	Number of hours at 10°F	Adults obtained
Cheopis	12	24	9
Astia	11	24	11
Cheopis	12	48	5
Astia	12	48	2

Natural enemies of fleas—*Tyroglyphidæ* have frequently been present in the breeding jars in countless myriads without interfering with the output of young fleas Swellengrebel (1913) noted a similar infestation of his breeding jars in East Java In the present experiments, while precautions were taken to exclude ants, the only enemy of the developing flea which gave trouble was a species of wingless book louse (Fam *Psocidæ*) Book lice were frequently seen inside flea pupa cases, and they had obviously devoured the contents One damaged cocoon contained four eggs of the book louse, and a newly hatched louse was seen alongside Ants have made off with eggs from unplugged tubes Ants can also deal skilfully with adult fleas they have frequently swarmed into a lampglass containing many fleas, which they have rapidly killed and carried off Spiders can also prey on adult fleas On one occasion a small spider gained access to a lampglass containing 15 young active *cheopis*, and 13 of these were found dead, with the limbs amputated and the bodies cut open

SUMMARY

The three Indian rodent *Xenopsylla* readily feed on man in the absence of a more suitable host, even at temperatures of over 80°F

No striking difference has been demonstrated in the longevity of starved individuals of the three species

Observations on the life history of the three species are recorded in detail It appears that under Bombay conditions the hot weather is least favourable for *brasiliensis*, and the cold season is least suitable for *astia*, while *cheopis* thrives at all periods The developing stages of the fleas may survive temporary exposure to extreme conditions, e g , temperatures of 98 4°F and 40°F respectively for periods of 24 hours

Some natural enemies of rat-fleas have been noted

Rao Bahadur G D Chitre has given valuable assistance in obtaining the material for these studies

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OBSERVATIONS ON RAT-FLEAS AND THE TRANSMISSION OF PLAGUE

Part IV

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Mixed flea experiments, second series

THE mixed flea experiments previously reported, designed to allow comparison of the transmitting powers of fleas infected on the same septicæmic animal, proved inadequate on account of the small number of positive results It was decided to repeat the experiments using a larger number of fleas, and to omit the individual feeding This partially precluded the use of the same material for study of the blocking phenomenon

In this series therefore the technique is as follows A Madras rat is inoculated cutaneously with plague and is introduced to a flea-proof cage Next day 72 fleas, twelve each female and male *cheopis, astia* and *brasiliensis,* are added When the rat dies it is examined, and if the signs of plague are considered satisfactory, the fleas are collected They are sorted out according to species and sex, and added to cages containing healthy rats, each group to one rat or six rats in all These rats are examined as they die or when they are killed three weeks later The surviving fleas are collected ten to fourteen days after death of the inoculated rat and tested for plague infection individually by teasing up the proventriculus and stomach in saline and culturing the emulsion If a rat dies before the fleas are required for culture, the fleas are collected and put on another healthy rat so that, on occasion, a second and even a third transmission in series with the same small number of fleas has been obtained

Thirty-two of these experiments have been completed, and for analysis of the figures obtained they have been divided into two groups, viz, twenty in

the off season at a mean temperature of over 80°F, and twelve in the plague season at a mean temperature ranging between 68·8° and 76·8°F. Table I gives details of each experiment in turn, including the number of infected fleas employed and the successful transmissions obtained. In Table II the results of the cultural tests are shown. In the off season, thirteen of the twenty experiments were completely negative, while in the plague season transmission was obtained, with one or more species, in eleven out of the twelve. The last column of Table II gives the *percentage of successful transmissions for each* group of fleas. As regards the off season, all species actually transmitted, and as five out of the eight successes were with *bradhensis*, this species has been the most regular transmitter of the three at temperatures over 80°F. With

TABLE I

Mixed flea experiments, second series, 1929-30

Number of experiment	Date of inoculation	Temperature and humidity		Number of experiment	Date of inoculation	Temperature and humidity	
		Av. T	S.D.			Av. T	S.D.
146	July 12	81·1	183	166	Nov. 9	82·3	309
147	„ 20	81·8	197	167	„ 16	80·8	334
149	„ 27	82·1	205	169	„ 30	80·3	·398
150	Aug. 3	82·5	235	170	„ 30	80·3	·398
152	„ 10	82·8	235	171	Jan. 1	73·1	·331
154	„ 17	80·1	183	173	„ 11	72·4	213
155	„ 24	81·6	231	174	„ 18	72·2	265
156	„ 31	82·6	283	176	„ 25	74·3	·265
157	Sept. 7	81·7	·237	178	Feb. 1	68·8	237
158	„ 14	82·6	235	179	„ 1	68·8	237
159	„ 21	84·5	268	180	„ 8	72·5	302
160	„ 28	82·6	216	181	„ 8	72·5	302
161	Oct. 5	82·4	254	182	„ 15	75·0	374
162	„ 12	83·4	259	183	„ 15	75·0	374
164	„ 26	81·7	355	184	„ 22	76·8	·358
165	Nov. 4	82·5	344	185	„ 22	76·8	·358

The temperature and humidity figures are the averages for the seven days following the inoculation

TABLE I—*contd*

Experiment	146	147	149	150	152	154	155	156	157	158
cheopis female— Infected fleas used	12	12	12	14	8	8	12	8	2	11
Transmissions in series 1										
cheopis male— Infected fleas used	9	12	10	12	12	11	6	12	10	12
Transmissions in series 1										
astia female— Infected fleas used	9	10	12	9	11	9	8	12	12	8
Transmissions in series 1							13			
astia male— Infected fleas used	11	11	12	10	8	10	8	11	10	10
Transmissions in series 1							15			
brasiliensis female— Infected fleas used	10	10	11	11	11	11	11	11	11	13
Transmissions in series 1										
brasiliensis male— Infected fleas used	12	11	7	2	12	9	8	9	10	12
Transmissions in series 1					15				11	

Note —The number indicating successful transmissions indicates the number of days elapsing between death from plague of inoculated and test rats or successive test rats

the cold weather group, the first two experiments provided transmissions with all three species Later on *astia* gave no further positives, while *cheopis* and *brasiliensis* were frequently successful The total transmissions with one or both sexes, and counting the first in series only, was seven with *cheopis* and ten with *brasiliensis* With regard to the sex of the fleas it is very striking that the males were more often successful than the females The number of second and third transmissions in series indicates a balance in favour of *brasiliensis* as the most regular transmitter It may be added that the diagnosis of plague in the case of transmissions was generally quite clear from post-mortem examination and the examination of smears, an occasional case in which there was room for doubt being confirmed bacteriologically

The increased proportion of transmissions in the plague season did not apparently depend on certain factors which have hitherto been regarded as

TABLE I—*contd*

Experiment	159	160	161	162	164	165	166	167	169	170
cheopis female—										
Infected fleas used	12	12	12	10	11	12	12	12	11	12
Transmissions in series 1										
cheopis male—										
Infected fleas used	8	11	12	11	12	12	12	12	11	11
Transmissions in series 1		11								
astia female—										
Infected fleas used	11	11	12	11	12	12	8	12	11	10
Transmissions in series 1										
astia male—										
Infected fleas used	12	11	9	10	12	10	11	12	11	12
Transmissions in series 1										
brasiliensis female—										
Infected fleas used	12	12	11	11	12	11	9	11	11	9
Transmissions in series 1							5			9
brasiliensis male—										
Infected fleas used	1	11	9	9	12	12	12	8	10	11
Transmissions in series 1			7							
Transmissions in series 2			5							

Note —The number indicating successful transmissions indicates the number of days elapsing between death from plague of inoculated and test rats or successive test rats

of prime importance, viz, (*a*) the proportion of fleas infected, (*b*) the survival of the infected fleas, and (*c*) the survival of the plague bacillus in the infected fleas The proportion of the original fleas recovered, firstly from the inoculated rats, and subsequently from the test rats as shown by the numbers available for culture, is very much the same in the two groups Culture showed that a very large proportion of the fleas still harboured the plague bacillus ten to fourteen days after death of the inoculated rats, even in the off season The figures are not strictly comparable, as in the case of transmissions the fleas had one or more further opportunities of becoming infected In the case of male *brasiliensis*, where there were more transmissions at both seasons than in the other groups, there was actually a smaller proportion of infected fleas in the

TABLE I—*concld*

Experiment	171	173	174	176	178	179	180	181	182	183	184	185
cheopis female—												
Infected fleas used	12	11	11	11	11	5	10	10	12	12	11	11
Transmissions in series 1	6											
cheopis male—												
Infected fleas used	11	11	12	12	12	5	9	10	11	12	11	11
Transmissions in series 1		5	11	3	10	10			20			
Transmissions in series 2		6	6	9	5							
astia female—												
Infected fleas used	9	11	9	11	11	5	6	8	12	12	12	12
Transmissions in series 1	13	9										
astia male—												
Infected fleas used	10	11	11	12	11	5	11	10	11	12	11	12
Transmissions in series 1												
brasiliensis female—												
Infected fleas used	12	10	12	12	12	5	7	10	12	10	11	11
Transmissions in series 1	9	7							16			
brasiliensis male—												
Infected fleas used	4	11	12	12	12	5	12	12	10	12	12	12
Transmissions in series 1	11	8	6	5	7			12		9	9	16
Transmissions in series 2		4	3	3	4					10	6	
Transmissions in series 3			4	4	3							

Note —The number indicating successful transmissions indicates the number of days elapsing between death from plague of inoculated and test rats or successive test rats

plague season The cultures did not suggest that the infection was of a lesser degree in the off season Therefore, the change in climatic conditions, the other factors remaining similar, allowed a larger proportion of the infected fleas to become capable of transmitting the infection The conclusion is that the factor affected is the blocking phenomenon This presents an elusive and fascinating problem The blocking is rarely detected in infected fleas Whether because only a small proportion ever become blocked, or because the condition is transient, or because it is a rapidly fatal event, is not at all clear During identification of the fleas, firstly before they were put on the test rats, and later when

collected for culture, a small proportion showed some evidence of plague growth in the œsophagus. Only two blocked fleas—a female *astia* and a female *brasiliensis*—were recognized, details of which will be given later.

One point has attracted attention and seems to deserve further study, namely the occurrence, not of a pure infection with the plague bacillus, but a mixed infection with one or more coccal organisms in large numbers along with *B. pestis*. As will be shown later, the small number of blocked fleas available provided examples of a similar mixed infection. The cultures (Table II) showed

TABLE II

Mixed flea experiments, second series

TOTAL FLEAS DEALT WITH				RESULT OF CULTURES				TRANSMISSIONS	
Species and sex	Original number	INFECTED FLEAS		*B. pestis* PRESENT		COCCI ALONG WITH PLAGUE		Nos. 1 IN SERIES ONLY	
		Used	Cultured	No.	Per cent	No.	Per cent	No.	Per cent
Off season 20 experiments—									
cheopis female	212	215	113	39	34·5	8	20·5	0	0·0
cheopis male	211	218	127	79	62·2	16	20·3	1	5·0
astia female	210	210	127	62	48·8	10	16·1	1	5·0
astia male	210	211	129	61	19·6	9	14·1	1	5·0
brasiliensis female	210	218	120	51	42·5	16	31·4	2	10·0
brasiliensis male	225	190	98	70	71·4	11	15·7	3	15·0
TOTAL	1,428	1,262	714	365	51·1	70	19·2	8	6·7
Plague season 12 experiments—									
cheopis female	144	127	84	52	61·9	7	13·5	1	8·3
cheopis male	141	127	77	61	79·2	2	3·3	6	50·0
astia female	144	118	91	53	58·2	0	0·0	2	16·6
astia male	144	127	83	25	30·1	4	16·0	0	0·0
brasiliensis female	144	124	79	61	77·2	3	4·9	3	25·0
brasiliensis male	144	126	66	43	65·2	0	0·0	9	75·0
TOTAL	864	749	480	295	61·5	16	5·4	21	29·2

Note.—The term 'infected fleas' indicates the fleas recovered from recently dead inoculated rats. It is not presumed that every one harboured the plague bacillus.

a very variable state of affairs in this respect In most of the positive cultures a pure growth of *B pestis* was obtained In some experiments as many as 70 or 80 per cent of the fleas showed a mixed infection In others only an occasional flea was so affected, the others being sterile or showing a pure plague infection Taking the two groups of experiments, it is seen that on the whole there was more of the mixed infection in the off season

The mixed flea experiments provided some information on an important variant in the habits of rat-fleas It is known, for example, that *Ceratophyllus* fleas may spend a large part of their lives apart from their hosts, but there is no comparative information of this type regarding the *Xenopsylla* When the fleas were collected from the dead inoculated rats, and later from the test rats, it was noted whether they were found on the host or in the layer of bran and sand spread on the floor of the cages The consolidated results are given in Tables III and IV The former indicates that the fleas had not vacated

TABLE III

Location of live fleas recovered from transmission cages containing recently dead inoculated rats

Species and sex	*cheopis*		*astia*		*brasiliensis*	
	female	male	female	male	female	male
Off season						
Number	218	220	211	213	219	196
Per cent on carcass	61 9	65 0	42 7	41 8	63 9	77 5
Plague season						
Number	130	128	119	127	127	127
Per cent on carcass	23 8	32·0	42·9	44 9	70 1	74 0

the carcass It is customary to draw a graphic picture of the departure of fleas from a plague-stricken rat One writer (Mason, 1915) states that the fleas begin to leave the dead rat in 15 seconds and that all have departed in two hours and a quarter In the pit experiments it was found that, although there were live rats close at hand, active fleas could be regularly recovered from the carcasses, even when they had begun to putrify It is possible that in a colder climate, with more rapid cooling of the carcass, this feature may not be evident The small figures now available suggest that, in the plague season, *cheopis* fleas had more rapidly deserted the carcass With the live rats, a very large proportion of all the fleas, even the females which must make regular

trips for egg laying, was found on the host It is not impossible that the wandering may be largely nocturnal, but the figures suggest that the three species show no marked difference in the proportions on host and abroad respectively

TABLE IV

Location of live fleas recovered from transmission cages containing live rats and fleas of one sex and species only

Species and sex	*cheopis*		*astia*		*brasiliensis*	
	female	male	female	male	female	male
Off season						
Number	117	132	132	135	126	105
Per cent on carcass	81·6	96·2	72·0	77·7	77·0	92·1
Plague season						
Number	85	69	82	85	79	65
Per cent on carcass	91·8	95·7	86·6	61·7	81·0	87·7

The pit experiment

The details of this method were described in the introductory paper The epizootic among Madras rats continued without a break in both *cheopis* and *astia* pits Up to December 23rd 576 rats had died of plague in the *cheopis* pit, and 618 in the *astia* pit There had been two survivors in each pit for from 134 to 186 days The flea population remained absolutely pure, several hundred fleas were examined weekly, and these samples showed only the respective species in each pit *Tyroglyphidæ* had not got a footing in either pit, and ticks and lice were only occasionally seen Beetles, both *Calandra* and *Tribolium* species and their larvæ, were present in considerable numbers during the last three months However, the precautions taken had prevented the infestation with Acarines which would soon have resulted in a seething mass of mites The flea population did not again become excessive The plague casualties from April onwards are shown in Table V The post-mortem appearances of the carcasses from the two pits was identical The location of the buboes in 400 rats infected by *cheopis* and a similar number by *astia* is given in Table VI The figures are taken from a regular sequence of deaths from plague from May 1st onwards There is a remarkable similarity in the two groups

All rats were removed on December 23rd, most of the fleas found on them being returned to the pits Apart from daily attention to the tanglefoot on

TABLE V

Experimental epizootic with Madras rats in Bombay, 1929

Month	Mean temperature	Approximate saturation deficiency	X *cheopis* pit		X *astia* pit	
			Deaths from plague	Average number of days	Deaths from plague	Average number of days
April			70	3·7	50	4·3
May	87·9	405	80	4·0	76	4·1
June	82·9	211	69	4·7	66	4·8
July	81·6	190	70	4·5	77	4·6
August	81·8	219	56	4·5	57	5·2
September	82·6	240	41	4·9	59	4·3
October	82·6	285	59	4·4	61	4·3
November	80·3	320	67	4·5	67	3·6
December	74·8	272	48	4·2	39	3·9
		TOTAL	560	4·4	572	4·3

TABLE VI

Situation of buboes in plague rats infected by X cheopis *and* X astia

Infecting fleas	Number of rats examined.	BUBOES DETECTED		Single
		Nil	Multiple	
X *cheopis*	400	114	82	204
X *astia* ..	400	131	73	196

Site of the single buboes

	Submaxillary	Cervical	Axillary	Pelvic	Others
cheopis group	109	18	44	32	1
astia group	80	19	49	48	0

the walls, the pits were left untouched until January 3rd when six Madras rats were added to each pit Three weeks later the rats were all alive, so it was concluded that fleas alone had failed to carry the infection over the gap

trips for egg laying, was found on the host It is not impossible that the wandering may be largely nocturnal, but the figures suggest that the three species show no marked difference in the proportions on host and abroad respectively

TABLE IV

Location of live fleas recovered from transmission cages containing live rats and fleas of one sex and species only

Species and sex	*cheopis*		*astia*		*brasiliensis*	
	female	male	female	male	female	male
Off season						
Number	117	132	132	135	126	105
Per cent on carcass	81·6	96·2	72·0	77·7	77·0	92·1
Plague season						
Number	85	69	82	85	79	65
Per cent on carcass	91·8	95·7	86·6	61·7	81·0	87·7

The pit experiment

The details of this method were described in the introductory paper The epizootic among Madras rats continued without a break in both *cheopis* and *astia* pits Up to December 23rd 576 rats had died of plague in the *cheopis* pit, and 618 in the *astia* pit There had been two survivors in each pit for from 134 to 186 days The flea population remained absolutely pure, several hundred fleas were examined weekly, and these samples showed only the respective species in each pit *Tyroglyphidæ* had not got a footing in either pit, and ticks and lice were only occasionally seen Beetles, both *Calandra* and *Tribolium* species and their larvæ, were present in considerable numbers during the last three months However, the precautions taken had prevented the infestation with Acarines which would soon have resulted in a seething mass of mites The flea population did not again become excessive The plague casualties from April onwards are shown in Table V The post-mortem appearances of the carcasses from the two pits was identical The location of the buboes in 400 rats infected by *cheopis* and a similar number by *astia* is given in Table VI The figures are taken from a regular sequence of deaths from plague from May 1st onwards There is a remarkable similarity in the two groups

All rats were removed on December 23rd, most of the fleas found on them being returned to the pits Apart from daily attention to the tanglefoot on

TABLE VII

Comparative transmission experiments with fleas from epizootic pits

Serial numbers of experiments	Date	Number of fleas in each case	Successful transmissions with number of intervening days			
			X cheopis		*X astia*	
			female	male	female	male
103—106	25-4-29	50	—	—	+13	—
107—110	26-4-29	100	+4	—	+5	—
111—114	7-5-29	50	+6	—	—	—
115—118	9-5-29	50	+7	—	+7	—
120—122	31-5-29	50	—	—	—	—
123—126	5-6-29	50	—	—	—	—
131—134	12-6-29	50	+7	+8	+12	—
135—138	20-6-29	50	+9	—	+10	—
142—145	4-7-29	40	+9	+14	+12	—
TOTAL		490	6	2	6	0
AVERAGE NUMBER OF DAYS			7	11	10	—

Note —The first four experiments have been reported previously but are included to show the full series One of the experiments with 50 male *astia* was abandoned

showed dark material in the œsophagus, which in many cases had disappeared a few hours later The great majority of these fleas, when tested on living rodents, succeeded in getting a blood meal and failed to infect the animal Diagnosis of the blocked flea in the capillary tube is not at all straightforward The most suspicious appearance has only been appreciated after much practice A dark mass distends the œsophagus as far as the third thoracic segment at least, and this widens gradually as it passes back to the proventriculus This tapering causes an obliteration of the angle between the œsophagus and the proventriculus, giving an appearance which at least frequently accompanies the blocked condition Russian workers (Burchkov and Borzenkov, 1929) have recently drawn attention to a new means of diagnosing blockage of a plague-infected flea by observation of the proventriculus isolated by dissection Normally the external appearance of this structure is nodular or mulberry-like, while in blocked fleas the exterior appears smooth This has been confirmed

by examination of a few blocked fleas which were dissected The Russian experience indicated that blocked fleas diagnosed by this method generally had no food residue in the stomach or rectum It is considered that under Bombay conditions a blocked flea would die before it became so empty

The available information regarding the behaviour and fate of the few blocked fleas detected will be given in detail This study is of a laborious nature, as a separate animal was used for feeding each flea suspected to be blocked A large number, in addition to those detailed, was fed individually, the only interesting result being the occurrence of infection by a flea which obtained a blood meal at the same bite This case of infection by a partially blocked flea is considered of importance as such an individual might obviously live much longer than a completely blocked flea The details regarding this specimen are as follows —

23-10-29 *Chcopis*, female, from pit, showed stout tapered clot in œsophagus Applied to a mouse, it fed slowly and red blood remained in the œsophagus after the feed The mouse remained healthy Next day it was applied to another mouse and sucked vigorously for several minutes Red blood was seen to surround the black residue in the stomach, but the proventriculus remained black and red blood was seen in the œsophagus after the feed *The mouse died of plague four days later* The flea was not fed again and was dead 48 hours after the last feed It was not, of course, suspected as a transmitter at the time of the feeding

The blocked fleas are described in the order they were met with The temperature figures refer to the dry and wet bulb readings at 9 A M on the date given The following abbreviations are used 3T = third thoracic segment, œs = œsophagus, pv = proventriculus

1 29-4-29, d b 86, w b 79, *cheopis*, male, with black clot in œs Applied to a rat, it inserted the biting parts four times in five minutes, and sucked vigorously but drew no blood The rat survived The flea was applied to a mouse immediately afterwards and it bit but failed to draw blood The mouse survived The flea died the same day

2 11-6-29, d b 86, w b 80, *astia*, male, with slender black clot along œs equal in length to pv Applied to a mouse it sucked vigorously for three minutes and red blood was seen to reach pv once The mouse survived Next day the flea filled rapidly when applied to another mouse Smear showed many plague-like bacilli

3 8-7-29, d b 83, w b 80, *astia*, female, with stout tapering mass in œs reaching ant 3T Applied to a mouse it inserted in several places and tried different levels over a period of five minutes Red blood was seen to reach pv once After the feed red blood could be seen in œs only The mouse survived The flea was dead next day Dissected it showed brown clot in œs Culture gave no *B pestis* but many cocci

4 17-7-29, d b 83, w b 78, *astia*, female, with tapered clot in œs reaching 3T Applied to a mouse it sucked for two minutes but blood reached as far as pv only After the feed red blood was seen in œs only The mouse survived The flea was dissected at once and showed a tough clot in stomach and pv and red blood in œs Culture gave no *B pestis* but many cocci

5 6-9-29, d b 80, w b 76, *astia*, female, with an empty œs but a dense black stomach and pv Applied to a rat, red blood reached as far as pv only After the

feed red blood was seen in œs and ant pv only The rat survived The flea died the same day

6 10-9-29, d b 79, w b 75, *astia*, female, showed a conical mass obscuring the pv Applied to a rat it sucked for five minutes and vibratile red blood was seen in œs After the feed no red blood could be seen *The rat died of plague five days later* The flea was dead next day Culture showed scanty *B pestis* and many *cocci*

7 10-9-29, d b 79, w b 75, *astia*, female, with stout tapered clot reaching 3T It had refused to feed on the previous day when the appearance was similar Applied to a rat it bit four times in five minutes and red blood was seen in the œs each time The rat survived Next day (d b 80, w b 75), the appearance was still the same and when applied to a mouse it behaved as before and a minute droplet was seen to escape from the proboscis at the last withdrawal After the feed the pv was still black and red blood was visible along the whole œs *The mouse died of plague five days later* The flea was dead next day Dissection showed that the nodular appearance of the pv was obliterated Culture gave many *B pestis* and many *cocci*

8 18-9-29, d b 82, w b 77, *astia*, female, with black clot in œs reaching 3T Applied to a rat it sucked vigorously for five minutes and reinserted twice when disturbed At the last withdrawal a droplet was seen to leave the proboscis After the feed the œs still seemed distended with red blood The rat survived The flea was dead next day Smear showed many *cocci* but no plague-like bacilli

9 20-9-29, d b 82, w b 76, *astia*, female, with black tapering clot in œs This flea had refused to feed on the two previous days when the appearance was similar Applied to a mouse it sucked for five minutes A broad band of red blood was seen in œs but did not pass the pv The mouse survived The flea was dissected next day It showed a smooth pv which contained an adherent clot Culture gave many *B pestis* and scanty *cocci*

10 10-10-29, d b 81, w b 77, *astia*, female, with tapered clot as far as 3T Applied to a mouse it sucked for two minutes and a broad band of red blood was seen in œs After the feed a little red blood was seen just in front of pv The mouse survived Next day the flea refused to feed on first a mouse and then a rat It was then dissected and showed some clot in œs but the pv had a normal nodular appearance Culture showed no *B pestis* but many *cocci*

11 30-10-29, d b 83, w b 74, *cheopis*, female, with tapered clot as far as ant 3T It had been partially blocked on the two previous days Applied to a mouse it bit and red blood replaced the clot as far as the pv The mouse survived The flea was alive next day It was dissected and showed clot in œs but the pv was normal Smear showed many plague-like bacilli and culture gave pure *B pestis*

12 30-10-29, d b 83, w b 74, *cheopis*, female, with dark clot along the whole œs Applied to a mouse it bit at once and red blood replaced the clot as far as the pv After the feed a trace of red blood could be seen near the pv The mouse survived The flea was alive next day Dissection showed a smooth pv Smear gave many plague-like bacilli and culture a pure growth of *B pestis*

13 7-11-29, d b 82, w b 79, *cheopis*, female, with the pv obscured by a blurred mass Applied to a mouse it sucked for several minutes and a broad band of red blood was seen in œs After the feed red blood was seen in œs and ant pv only The mouse survived Next day the flea appeared normal and the following day it was dead

14 18-11-29, d b 82, w b 76, *astia*, male, with a long tapered clot in œs reaching ant 2T Applied to a mouse it sucked for five minutes, blood passing as far as the pv only After the feed red blood was seen in the anterior part of pv and along the œs as far as ant 3T *The mouse died of plague four days later* The flea was dead next day Dissection showed that the nodular appearance of the pv was obliterated

15 21-11-29, d b 78, w b 65, *brasiliensis*, female, with a large blurred-looking pv Applied to a mouse it sucked for some minutes and red blood reached the pv twice After the feed no red blood could be seen The mouse survived The flea was dead next day On dissection the pv was nodular Smear showed many plague-like bacilli

16 1-12-29, d b 83 w b 73 *cheopis*, female, after two days starvation still showed a short clot in œs Applied to a mouse it bit several times and struggled violently A droplet left the proboscis at the last withdrawal After the feed red blood could be seen in the anterior pv and along part of œs *The mouse died of plague six days later* The flea was dead next day and dissection showed a smooth pv

The smears referred to were prepared by teasing up the proventriculus and stomach in a little saline and smearing out the emulsion Material for culture was taken from the same emulsion In all these cases only the one flea was fed on each animal, and the surviving rats or mice were killed and examined after the usual three weeks None of the survivors showed signs of resolving or recovered plague

These fleas were chiefly obtained from the pits The exceptions were Nos 6 and 15, the former being detected among the fleas of mixed flea experiment 155 on the day that the test rat died of plague, while the latter belonged to experiment 166 being found blocked five days after the test rat died of plague These two suggest that a flea once blocked may remain dangerous, at least at intervals, over a period of some days On the other hand, the four blocked fleas which transmitted were all dead on the day following the infecting bite Further study of blocked fleas is required The chief interest of the results so far is the production of blocked female and male *astia* at a mean temperature close to 80°F and the transmission of infection by some of these at a single bite

Virulence of the plague bacillus in the flea

One experiment was designed to demonstrate any loss of virulence on the part of the plague bacillus as a result of its sojourn in the flea At the conclusion of mixed experiment 167, in which no transmission occurred, each flea was dissected and the usual emulsion prepared A sample was taken for culture and the remains of the emulsion in each case was mixed with 0 2 c c saline and injected subcutaneously into a Madras rat The rats were examined as they died, the survivors being killed after the usual three weeks The results are given in Table VIII Out of 40 fleas examined, 28 gave *B pestis* on culture Of the rats corresponding to the negative cultures all remained healthy Of the 28 rats corresponding to the positive cultures, 23 died of plague between the second and seventh days after injection The remaining five showed local abscesses at the site of inoculation three weeks later, and in three of these plague bacilli were isolated from the pus In particular, it is seen from the table that in the case of *astia* and *brasiliensis* there is no suggestion of a diminished virulence of the bacillus ten days after infection of the fleas

TABLE VIII

Virulence of plague bacilli ten days after infection of fleas

(Fleas of mixed experiment 167)

No	Fleas Species	Sex	Cultures *B pestis*	Days after inoculation when rat died of plague						Condition of survivors
				2	3	4	5	6	7	
1	*cheopis*	F	—	—	—	—	—	—	—	Normal
2	„	F	—	—	—	—	—	—	—	„
3	„	F	—	—	—	—	—	—	—	„
4	„	F	+	—	—	—	—	—	—	Abscess, B p +
5	„	F	—	—	—	—	—	—	—	Normal
6	„	F	+	—	—	+	—	—	—	—
7	„	F	—	—	—	—	—	—	—	Normal
8	„	F	—	—	—	—	—	—	—	„
9	„	F	—	—	—	—	—	—	—	„
10	„	F	—	—	—	—	—	—	—	„
11	„	M	+	—	—	—	+	—	—	—
12	„	M	+	—	+	—	—	—	—	—
13	„	M	+	—	+	—	—	—	—	—
14	*astia*	F	+	—	+	—	—	—	—	—
15	,	F	+	+	—	—	—	—	—	—
16	,	F	+	—	+	—	—	—	—	—
17	„	F	+	—	+	—	—	—	—	—
18	„	F	—	—	—	—	—	—	—	Normal
19	„	F	+	—	+	—	—	—	—	—
20	„	F	+	—	+	—	—	—	—	—
21	,	F	+	—	—	—	—	—	+	—
22		F	+	—	+	—	—	—	—	—
23	,	F	+	—	—	+	—	—	—	—
24	„	M	+	—	—	—	—	—	+	—
25	„	M	—	—	—	—	—	—	—	Normal
26	„	M	+	—	+	—	—	—	—	—

TABLE VIII—*concld*

No	Fleas Species	Sex	Cultures *B pestis*	Days after inoculation when rat died of plague						Condition of survivors
				2	3	4	5	6	7	
27	*astia*	M	+	—	—	—	—	—	—	Abscess, Bp +
28	„	M	+	—	—	—	—	+	—	—
29	„	M	—	—	—	—	—	—	—	Normal
30	„	M	+	—	—	—	—	—	—	Abscess
31	„	M	—	—	—	—	—	—	—	Normal
32	*brasiliensis*	F	+	—	+	—	—	—	—	—
33	„	F	+	—	+	—	—	—	—	—
34	„	F	+	—	—	+	—	—	—	—
35	„	F	+	—	+	—	—	—	—	—
36	„	F	+	—	—	—	—	—	—	Abscess, Bp +
37	„	F	+	—	—	+	—	—	—	—
38	„	M	+	—	—	+	—	—	—	—
39	„	M		—	—	—	+	—	—	—
40	„	M	+	—	—	—	—	—	—	Abscess

DISCUSSION

Previous observations on this subject have been discussed by Hirst in his Memoir and it is unnecessary to repeat the story. The recently published compilation of the Medical Research Council (Petrie, 1929) states that the rôle of *X astia* is unsettled and that there is no detailed information regarding *X brasiliensis*. It concludes 'To sum up, the prevalence of bubonic plague is dependent on the prevalence of rat plague and this in turn is governed by the climatic factors—temperature and relative humidity—which influence the developmental stages of the rat-flea and the multiplication of the plague bacillus within the flea'.

X cheopis, *X astia* and *X brasiliensis* have all been found capable of transmitting plague. The proportion of fleas harbouring the plague bacillus which ever become capable of conveying the infection is not large, and may vary greatly with changes in climatic conditions. When two species of flea are of unequal value as transmitters, the difference will be the more marked the fewer the fleas used in comparative transmission experiments. If many

fleas are used the difference may be largely concealed The mixed flea experiments with a dozen fleas or less in each case indicate that *astia* is a much less regular transmitter than either *cheopis* or *brasiliensis* The blocking phenomenon has, however, been observed in both sexes of *astia* fleas, and continuous transmission experiments with *astia* fleas only, have been successful, both with a very large flea population and with a proportion of fleas to rats more closely approaching that met with in nature

Under experimental conditions *cheopis* and *brasiliensis* were much more successful transmitters during January and February in Bombay than at other times of the year *X astia* was apparently not much more effective in these months than at other times The question of the sex of the flea in connection with its transmitting power is not easy to answer From the mixed flea experiments it would have seemed reasonable to conclude that, of the six groups, male *cheopis* and male *brasiliensis* are much the most regular transmitters With the pit fleas, however, the cage experiments show female *cheopis* and female *astia* to be much more effective than their respective males Male *astia* fleas have been found of low value as transmitters in all the experiments The finding of an individual blocked male *astia* capable of infecting with a single bite was particularly fortunate The experiments of Hirst, of Taylor and Chitre and of Goyle all gave fewer transmissions with *astia* than with *cheopis*, and in most of these the number of fleas employed was larger than in the mixed flea experiments The conclusion is that although both sexes of the three species are potential transmitters of plague, yet the proportion of infected *astia* which become capable of transmitting is much less than in the case of *cheopis* or *brasiliensis* In other words, where a specifically pure flea population is concerned, a higher *astia* index is required for the continuance of epizootic plague

The exact numerical value of the different species cannot yet be laid down The renewed epizootic in the *astia* pit provided some information regarding this species With a flea index of seven the epizootic could be restarted When the epizootic had ceased the flea index was found to be 3 2 Under the most favourable conditions the necessary *astia* index may lie between these two figures In nature a still higher index is probably required The mixed flea experiments in the plague season, taking both sexes of each species and counting the first transmission in series only, give the relative value as transmitters of *cheopis*, *astia* and *brasiliensis* as 1 0 3 1 7 respectively

In Bombay city the months of December, January and February are much the most favourable for experimental plague transmission In former years these months were noted for a great rise in the number of human plague cases The epizootic and epidemic being then thoroughly established, were able to proceed on much the same scale for another few months, when, judging by the results of cage experiments, the conditions are much less favourable for transmission In May the climate is hotter and drier (*see* Table V) and the abrupt decline in the number of human cases about the end of May was for

OBSERVATIONS ON THE NATURAL HISTORY OF *F BANCROFTI* IN DWELLINGS IN RELATION TO THE SYSTEMS OF DRAINAGE

Part VII

BY

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I INTRODUCTION
II MATERIAL AND TECHNIQUE
III RESULTS OBTAINED IN FIELD STUDIES
A Dwellings in relation to the systems of drainage in different endemic areas (Gangetic plain)
B Filarial incidence according to age, sex and signs in relation to systems of drainage
C Filarial incidence and occupation
D *Culex fatigans*
IV DISCUSSION OF RESULTS
V CONCLUSIONS

I INTRODUCTION

THE results of investigation into the filarial situation in Bihar and Orissa have revealed so far that the incidence of filariasis varies with the nature of arable terrain and that the *intensive* endemic areas are governed by the correlated factors of (1) terrain at the sea level, (2) arable nature of land where physical factors are such as to yield a staple crop like paddy, (3) urban or suburban population, (4) incidence of *F bancrofti*, (5) presence of *Culex fatigans*, (6) collection of water under insanitary surroundings (Korke, 1930 *a* and *b*)

The interest of a practical sanitarian would necessarily lie in the knowledge of the facts which aid in determining how and where to start preventive measures in a given endemic centre To elucidate these points the *intensive* study of a *dwelling* appears to be a guiding factor

Observations on a dwelling incorporate the study of infection in the definitive and intermediate hosts and of breeding conditions of the intermediate host

C fatigans is a mosquito domestic in habits and from the filarial viewpoint its breed feed and keep may be looked for in and around the dwelling which offers it the necessary opportunities

A dwelling may be situated in an urban, suburban or a village area The main point of difference between these areas is the density of population per square mile and the degree of sanitation by which the area is controlled

Sanitation from the filarial aspect depends on the mode and method of disposal of house water from a dwelling The methods of disposal can principally be classified under four systems, viz, cemented or 'pucca drain', non-cemented or 'kuccha' drain, cess pool or 'bowl' (mostly non-cemented), and absence of any system or a promiscuous way of disposing of the house water

It has been observed that parts of an urban or suburban area are not exclusively provided with one single system but combinations of all these systems occur, one emptying into another

The *inefficiency* in the proper working of any one of the systems leads to the accumulation of water and once this forms pools and is left standing, it favours the breeding of *Culex fatigans*

In addition to these sources of accumulation, there are artificial excavations like tanks, reservoirs, canals or wells, used for domestic and agricultural purposes near a dwelling

Considerations like the above suggest the study of the natural history of filariasis in a dwelling in relation to drainage system

II Material and technique

The material for this investigation was derived from families and sections of the population residing under different, or different combinations of, drainage systems (Fam, 82, Cases, 480, Group cases, 421)

The blood material was taken during the hours of 7 and 10 at night The embryos in the peripheral blood were identified as those of *bancrofti*, and the cases were classed as positive

In the case of dwellings investigated (1) the inmates were examined, (2) *Culex fatigans* was caught and dissected (females only), (3) mosquito larvæ were collected from the drainage system prevailing around the dwelling, the larvæ bred out at the laboratory at Gaya and the adults identified

In addition, mosquitoes were caught and dissected from areas at a distance from the actual site of investigation This was to ascertain the prevailing species of mosquito and to determine the filarial infection in the area

Signs were classified into affections of the male genitalia, termed 'scrotal,' and affections of the extremities such as fugitive and permanent œdemas in males and females, termed 'terminal' These signs were considered to be 'pathognomonic' of the filarial condition in an endemic area

Examination of the female population in a family was the most difficult part of the Inquiry The females generally belonged to the well-to-do classes of Hindus and Mohammedans and owing to the strict purdah conditions in these parts, the peripheral blood was taken from the hand protruded through a purdah Cautious and discreet inquiries were essential regarding the signs in the females For the sake of brevity in the table, ages up to 20 years are given as 'adolescent' males and females The nomenclature adopted is as follows —

C D, cement drain, N C D, non-cement drain, C P, cess pool, N S, absence of any system, R, reservoir, tank or canal, Pop, sections of the population, Grp, groups of population, Fam, families, Cas, cases, Post, cases positive, P C, percentage, Scrot signs, scrotal, Term, signs, terminal

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III Results obtained in field studies

A Dwellings in relation to the systems of drainage in different endemic areas (Gangetic plain)

Area 1, urban, Bihar, a large area next to Patna situated in richly cultivated land about 20 miles south of the Ganges and dotted with reservoirs Two cement drains run practically the entire length of the narrow main thoroughfare (over a mile) and empty into a large pit at the southern extremity of the town The dwellings situated on the southern sector of this drainage were investigated In other parts of the town and in the investigated suburbs like Sohdih and Soho, cess pool and other systems prevail

Details regarding incidence of infection in families and groups of the population and signs manifested under different systems of drainage are given under Table I

Dates of investigation, 4-2-1930 to 28-3-1930

Incidence—Bihar proper total cases, 193, positive, 41, per cent, 21 Sohdih —total cases, 30, positive, 4, per cent, 13 Soho —total cases, 18, positive, nil

Intensity of infection—Systems C D plus R, per cent, 50, C D plus C P, per cent, 37 5, C D, per cent, 19 3, N C D (cases few), per cent, 20, C P, per cent, 10 6, N S, per cent, 10

Frequency of signs—Systems C D plus R, scrotal, per cent, 50, C D scrotal, per cent, 39 4, terminal (male), per cent, 22 5, terminal (female), per cent, 26 C P, scrotal, per cent, 31, term, per cent, 11, term (fem), per cent, 5 C P plus R, scrotal, per cent, 16 6, term, per cent, 11 6, term

(fem), per cent, 9 3 (Parbalpur area is included in this system) The figures for the rest of the systems are low

History of a filarial family—For reference in the discussion of results I give particulars of one family in some detail The object is to show, what in all probability happens under the optimum conditions of infection

Family, Bihar, rich Mohammedans, house spacious, airy, approaching a mansion, isolated, with a large garden in the compound Inner courtyard especially of the Zenana quarters paved with stones having a well in the centre, disposal of water by paved drains In the near vicinity of the house stands a spacious reservoir always fed with water

Data, (1) male 55, positive, signs, chyluria, (2) male, 50, positive, attacks of lymphangitis, (3) male, 26, negative, signs, scrotal, (4) male, 24, positive, signs scrotal and chyluria, (5) male 24, positive, no signs, (6) male 24, positive, attacks of fever, (7) male, 12, negative, pains in the scrotum with attacks of fever, (8) male, 24, negative, no signs, (9) female, 45, negative, no history given, (10) female, 50, negative, œdema, arm The rest of the members were absent at the moment but there was a definite history of filariasis in some of them

Adults bred from larvæ collected from a cement drain in front of the house were all *C fatigans,* in fact the drain was teeming with the larvæ (7-4-1930)

Area 2, village, Parbalpur, about 10 miles from Bihar watered by reservoirs, system —cess pool, general information under Table I, total cases, 62, positive, 11, per cent, 18, date, 25-2-1930, no mosquito work was done in this area

Area 3, urban, Gaya, system C D , general information under Table I, total cases, 56, positive, 11, per cent, 20, date, 23-3-1930 to 25-3-1930

Intensity of infection system C D , fam , 19 per cent, group, 20 per cent

Frequency of signs figures low

Area 4, village, Waziganj, absence of any system, general information under Table I, total cases (fam), 115, positive, 12, per cent, 10, date, 25-2-1930 to 27-2-1930

Frequency of signs figures low

Area 5, village, Jamhor, situated in a richly cultivated area, 60 miles west of Gaya, areas investigated, 2, Jamhor proper possessing partly all systems of drainage and Jambigha about 2 miles west on the Punpun, representing no system, general information, under Table I, date, 26-11-1929 to 4-12-1929

Incidence total cases (fam), 52, positive, 7, per cent, 13, total cases (grp), 66, positive, 17, per cent, 26 Infection at Jamhor proper is 22 per cent and at Jambigha (cases, 13, positive, 1), 7 per cent

Intensity of infection systems C D plus R , 37 8, C D plus N C D , 20, C D , 18, N C D , 17, C P plus N C D , 10, N S plus R , 9, C P , nil

Frequency of signs figures low

Table I

Showing incidence and signs of filariasis in relation to the systems of drainage

Area and systems	Type Pop	Tot Cas	Post	P C	Adult male				Adolescent male				Adult female			Adolescent female		
					Cas	Post	Scrot	Term	Cas	Post	Scrot	Term	Cas	Post	Term	Cas	Post	Term
1 Bihar—																		
C D	Fam	24	5	21	11	3	3	1	4	1	1		5	1	1	4		1
„	Grp	74	14	19	44	10	21	12	12	3	3	3	15	1	5	3		
N C D	Fam	5	1	20	3	1		3					2					
C P	Fam	35	3	9	11	1	1		7	1			10	1		7		
„	Grp	11	3	27	4	1	3		7	2	2	1						
„ Soho	Grp	18			11		7	3	5		1	1	1		1	1		
N S	Grp	10	1	10	7	1	5		3									
C D & R	Fam	10	5	50	7	5	3		1		1		2		1			
C D & C P	Grp	24	9	38	4	2	2	1	3	1	•		13	3	4	4	3	
C P & R Sohdih	Grp	30	4	13	10	2	2		6	1			12		2	2	1	
2 Paibalpur—																		
C P & R	Fam	41	7	17	18	4	6	5	5	1		1	13	2	1	5		
	Grp	21	4	19	14	4	2	1	7									
3 Gaya—																		
C D	Fam	31	6	19	13	4	1		6	1			8	1		4		
	Grp	25	5	20	9	3			6	1			7	1		3		

TABLE I—*concld*

Area and systems	Type Pop	Tot Cas	Post	P C	Adult male				Adolescent male				Adult female			Adolescent female		
					Cas	Post	Scrot	Term	Cas	Post	Scrot	Term	Cas	Post	Term	Cas	Post	Term
4 Wazirgunj—																		
N S	Fam	115	12	10	27	5	1		29	2			35	3		21	2	
5 Jamhoi—																		
C D	Fam	11	2	18	5	2	1	1					4		1	2		
N C D	Fam	12	2	17	4				3	1			1	1	1	1		
N S	Fam	5			1				2		1		1			1		
„	Grp	29	3	10	7	1	1		16	2			6					
C P	Fam	4			1		1		1				2		1			
C D & R	Grp	37	14	38	18	13	1		5				12	1		2		
C D & N C D	Fam	10	2	20	4	2	2	1	2				3		1	1		
C P & N C D	Fam	10	1	10	3		1	1	3	1			2		1	2		
6 Nawadah—																		
C D	Fam	28	4	14	5	1			12				8	3		3		
N C D	Fam	30	2	7	10	2	1		9				9			2		
C P	Fam	37	1	3	9	1		1	9				14			5		
C D & C P	Fam	21	3	14	5		3	1	2		1		9	2		5	1	

7 Rajauli—																		
C D	Gip	29	4	11	18	2			8	1			3	1				
N C D	Gip	27	3	11	15	2	3		11	1	1					1		
	Fam	20			3			2	4				8		1	5		
C P & N C D	Fam	3			1								1		1	1		
Chowkidars	Villages	38	2	5	38	2	3											
Namatand	Gip	48	5	10	10	4			9				13	1		16		
8 Tekari—																		
C D	Fam	5			3		1	1	1			1	1					
N C D	Fam	3			1			1					2					
N S & R	Fam	8			2			1	2				2			2		
C D & R	Fam	4	1	25	1				1				1			1	1	
N C D & R	Fam	4	2	50					3	2			1					
C P & N C D	Fam	4	1	25	2	1	1	1	1				1					

Area 6, suburban, Nawadah divided by the river into area proper and Par Nawadah, prominent systems cement drains in the former and cess pool in the latter, general information under Table I, date, 26-1-1930 to 2-2-1930

Incidence Nawadah cases, 61, positive, 7, per cent, 11 4, Par Nawadah cases 55, positive, 3, per cent, 5 4

Intensity systems C D plus C P, 11 2, C D, 14, N C D, 6 6, C P, 2 7

Frequency of signs figures low

Area 7, village, Rajauli about 20 miles south of Nawadah and presents features of the submontane conditions, the river divides the area proper from village Nematand, cement drains and cess pool systems predominate in the former and absence of any system in the latter, general information under Table I, date, 19-1-1930 to 23-2-1930

Incidence Rajauli general area police chowkidars, per cent, 5, Rajauli proper total cases, 79, positive 7 per cent, 8 8

Area Nematand total cases, 18, positive, 5, per cent 10

Intensity of infection system C D (Rajauli), per cent, 14, N S, per cent, 11

Frequency of signs figures low

Area 8, suburban, Tekari, total cases, 28, positive, 4, per cent, 14, general information, Table I, date, 20-12-1929 to 29-12-1929

B Filarial incidence according to age, sex and signs in relation to the systems of drainage

The data in this respect have been given under Table II The evidence is twofold It relates (1) to the frequency with which a particular system is present, and (2) to the frequency with which a high percentage of infection in age, sex, etc, and in signs is given by a particular system Percentage of infection out of 83 systems (single or in combination) represented in the table, C P, appears 25 times, C D, 22, N C D, 15, R, 14, and N S, 7

Percentage of signs in male scrotal out of 14 systems, C D, appears 4 times, C P, 4, N C D, 3, R, 2, and N S, 1, terminal, C P, appears 4, C D, 3, N C D, 3, R, 2, and N S, 1 Percentage of signs in female terminal, C P, 4 times, C D, 4, N C D, 3, and R, 2

SUMMARY

1 Cement drain and cess pool appear most frequently either singly or in combination

2 In the three systems represented singly, viz, cement drain, cess pool, and non-cement drain, cement drain takes a leading part both in the percentage of infection and in the frequency of signs

3 Cement drain in combination with reservoir, cess pool, or non-cement drain, show a higher degree of infection both in the peripheral blood and in the percentage of signs than the combination of other systems

TABLE II

Filarial incidence according to age, sex and signs in relation to the systems of drainage

Serial number	Systems of drainage	PERCENTAGES IN AGE AND SEX				PERCENTAGES IN SIGNS		
		MALES		FEMALES		MALES		FEMALES
		Adult	Adolescent	Adult	Adolescent	Scrotal	Terminal	Terminal
	System, Single—							
1	C D	23	14	16		20	11	11
2	N C D	14	6	4		3	16	6
3	C P	8	10	4		23	9	5
4	N S	14	7	7	4	9		
	System, Combinations—							
5	C D & R	70		6	33	15		5
6	C P & R	24	11	8	14	16	12	9
7	C D & C P	22	20	23	44	43	14	14
8	C D & N C D	(50)	Figures low			33	16	25
9	C P & N C D	16	25			20	20	30
10	N S & R		40				14	

C Filarial incidence and occupation

The details of information are given under Table III The system adopted for classifying the sections of the population is as follows —

1 Hindus, better class, Brahmin, Rajput, Kayastha, Babhan

2 Hindus, unclassified (want of information)

3 Mohammedans agriculturalists (petty), traders and servant class

4 Servant, domestic class Kahar, Kurmi, Mali, Dhobi, Hajjam, Dosadh Dom, Sweeper

5 Traders (petty) class Bania, Teli, Goala, Passi, Tamboli, Ironmonger, Julai, Kumhar, Mallah, Chamar, Kandu

6 Artisan class Lohar, Sonar, Barhi

7 Agriculturists Village chowkidars, Kurmi, Kalwar, Rajwar

Table II, system C D and C P classes, better represented, infection 27 and 25 per cent respectively Systems C D and N C D, C P and

TABLE III

Incidence of filariasis as shown by blood examination and in signs according to sex and occupation in relation to the systems of drainage

System and occupation	MALES				FEMALES				Total showing signs per cent
	Blood positive		Blood negative		Blood positive		Blood negative		
	Show signs	No signs	Show signs	No signs	Show signs	No signs	Show signs	No signs	
System, C D—									
Hindus, Better Class	2	1	6	32		3	3	11	
,, Unclassified	2	9	13	17			1	2	
Mohammedans	1	1	16	9		2	2	8	
Servants, Domestic	1	8	1	21		2		20	
Traders, Petty	2	1	1	2		1	1	10	
Artisans			3	4				2	
Agriculturists			1	1					
TOTAL	11	26	41	86		8	10	56	27
System, C P—									
Hindus, Better Class	1			18				18	
,, Unclassified	1	2	6	5			1	4	
Mohammedans	3	2	7	11		7	3	20	
Servants, Domestic			1	2			1	1	
Traders, Petty			4	5			2	1	
Artisans			2	1					
TOTAL	5	4	20	42		7	7	44	25
System N C D—									
Hindus, Better Class	1	1		17				10	
Servants, Domestic			1	6				12	
Traders, Petty			1	2			1	1	
Artisans		1		5		1	1	3	
TOTAL	1	2	2	30		1	2	26	8

TABLE III—*contd*

System and occupation	MALES				FEMALES				Total showing signs per cent
	Blood positive		Blood negative		Blood positive		Blood negative		
	Show signs	No signs	Show signs	No signs	Show signs	No signs	Show signs	No signs	
System, N S—									
Hindus, Better Class	1	1	1	18					
„ Unclassified			4	3					
Mohammedans	1		1	12		1		4	
Servants, Domestic	2	4	1	13			1	18	
Traders, Petty	1	2	4	21		1	1	22	
Artisans		4		28		3		21	
Agriculturists		5	1	52		1	1	29	
Total	5	16	12	147		6	3	94	7
System, C D & R—									
Hindus, Better Class	1	1							
Mohammedans				2	1			1	
Servants, Domestic		3		3				4	
Traders, Petty		8	1	6		1		9	
Total	1	12	1	11	1	1		14	7
System, C D & C P—									
Traders, Petty			3	4		3		11	14
System, C D & N C D—									
Hindus, Better Class	1	1	1	3			1	3	30
System, C P & R—									
Hindus, Better Class		2	3	3				5	
Mohammedans		2	3	9		1	2	11	
Servants, Domestic	1		1	2					
Traders, Petty	1	6	8	19	1	1	1	10	
Total	2	10	15	33	1	2	3	26	23

TABLE III—*concld*

System and occupation	MALES				FEMALES				Total showing signs per cent
	Blood positive		Blood negative		Blood positive		Blood negative		
	Show signs	No signs	Show signs	No signs	Show signs	No signs	Show signs	No signs	
System, C P & N C D—									
Hindus Better Class	1		2	3			1	2	
Servants, Domestic	2	1	1	2				2	
Artisans				1			1	1	
TOTAL	3	1	3	6		.	2	5	40

N C D, classes not properly represented, infection 30 and 40 per cent respectively

D Culex fatigans

Accurate information on the bionomics and breeding conditions is still wanting in the case of this mosquito but the data so far collected are of the following nature —

Area, Bihar [infection in *C fatigans* in Bihar proper was found to be 33 per cent (Korke, 1930*a*)]

Collection and dissection of adults date, 8-2-1930 to 17-2-1930, temperature 15 5°–18°C wet and 22°–26°C dry bulbs, total *C fatigans* collected, 45, dissected, 27, positive, 5, per cent, 19, other species of mosquitoes found in the dwellings, none

Breeding-places (1) System C D, areas, 4, total mosquitoes bred, 162, females, 85, (2) system —C P, areas, 3, total mosquitoes bred, 283, females, 115, (3) pools of water total mosquitoes bred, 70, females, 38, (4) system N C D, total mosquitoes bred, 99, females, 42, all *Culex fatigans* From the first collection of larvæ to the first appearance of the imagines, time occupied, 3 to 4 days, the whole observation complete, 8 days Observations on 10 reservoirs in different areas of the town showed absence of breeding, date, 7-4-1930 and 8-4-1930, temp, 23 5°C wet and 35 5°C dry bulbs

Area, Gaya [infection in *C fatigans* in this area ranged between 14 and 30 per cent (Korke, 1930*a*)]

Breeding-places 28-3-1930, temp, 21 5°C wet and 35 5°C dry bulbs, system C D, 2 drains, total *fatigans*, 70, females, 30.

Area, Wazirgunj [nfection in *C fatigans* of this area was found to be 14 per cent (Korke, 1930*a*)]

Collection dwellings, 4, *C fatigans*, 36, *C bitæniorhynchus* 3, *C tritæniorhynchus*, 9, *A fuliginosus*, 5, no dissection

Breeding-places date, 28-2-1930 to 5-3-1930, temp, 17 5°–22°C wet and 29°–31°C dry bulbs, system C P, *C fatigans* bred, 16, fem, 5 Observation complete, 5 days

Area, Jamnoi [nfection in *C fatigans* of this area was found to be 7 per cent (Korke, 1930*a*)]

Collection date, 27-11-1929 to 4-12-1929, temp, 16 5°–22 5°C wet, 23°–25 5°C dry bulbs

Area, Jamhoi proper, dwellings, 5 (groups of population), *C fatigans* collected, 38, dissected, 18, positive, nil, dwellings, 13 (families), total mosquitoes collected, 202, dissected, 158, positive, 17, per cent, 11

Area, Jambigha, dwellings, 2 (groups of population), total *C fatigans* collected, 12, dissected, 11, positive, nil

Area, inspection bungalow (about a mile from the town) sparse pop, total mosquitoes collected, 19, dissected, 14, positive, nil Other species of mosquito found, total, 103, fem, 99, *A fuliginosus, A pallidus, A subpictus, A culicifacies, A hyrcanus, C tritæniorhynchus, C vishnui*

Other culicines dissected, all negative *C fatigans* was taken in abundance from the houses of families and the other species from the areas of Jambigha and Inspection Bungalow

Breeding-places larvæ collected from pools formed by well water when developed were all *C fatigans* Observation complete, 7 days

Area, Nawadah [nfection in *C fatigans* in this area was found to be 24 per cent (Korke, 1930*a*)]

Collection Nawadah, date, 27-1-1930 to 31-1-1930, temp, 14 5°–22°C wet, 20 5°–23 5°C dry bulbs

Dwellings, 4, *C fatigans*, 82, dissected, 61, positive, 7, per cent, 11 4

Collection Par Nawadah, dwellings, 7, *C fatigans*, 29, dissected, 15, positive, nil

Breeding-places Nawadah, system C D, *C fatigans*, 30 Par Nawadah, system C D, *C fatigans*, 36, N C D, *C fatigans*, 79, C P, *C fatigans*, 3; total females in the lot, 54 Observation complete, 2 to 5 days

Rajauli collection, date, 19-1-1930 to 25-1-1930, temp, 12°–16°C wet and 17 5°–20°C dry bulbs

Area proper, total *C fatigans* collected, 57, dissected, 21, positive, 1, other species collected from the dwellings, *A subpictus, C bitæniorhynchus, C tritæniorhynchus* and *A culicifacies* all in small numbers

Area Nematand, collected (mosquitoes scanty), total *C fatigans*, 11, dissected, 9, positive, nil

Breeding-places system C D (Rajauli) *C fatigans*, 76, females, 33

Area Tekari [infection in *C fatigans* in this area was found to be 19 per cent (Korke, 1930*a*)] Observation, date, 21-12-1929 to 28-12-1929, temp, 12°–16°C wet, 17°–19 5°C dry bulbs

Collection from dwellings 7, total *C fatigans*, 101, dissected, 83, positive, 8, per cent 10

Other species of mosquitoes total, 92, 1 *fuliginosus*, 56, 1 *subpictus*, 1 *pallidus*, *C vishnui*, males 5, of *C vishnui* only

The observations raise the question whether it is the numerical increase of the species bred in a particular system of drainage, or the longevity of the race probably kept virile by the moisture conditions of a particular system of drainage or reservoir which is the responsible factor

However, the evidence is corroborative of the fact (Korke, 1930*a*) that *C fatigans* will breed in these areas under summer and winter conditions, in cement drains, cess pools or any collection of dirty water provided the water is kept standing sufficiently long, for the females to lay eggs and pupæ to develop into adults

An important point, already referred to, is that to observe developmental forms of *F bancrofti* one should search for *C fatigans* within the dwellings

IV Discussion of results

The data on 901 cases have been presented for discussion The areas observed are more or less typical of the Gangetic plain

The first point that strikes one is that in a given area there are tracts or sub-areas which are not nearly so affected as are others Take for instance, Jamhor and Jambigha, Nawadah and Par Nawadah The physiographical, physical, social and economic factors are nearly the same, and yet there are differences in the degree of filarial infection

The cause for this variation may be looked for in the invertebrate host Fortunately one has to deal with only one species of host in Bihar and Orissa and it is easy to follow its trail *C fatigans* will breed in any shallow dirty collection of water under any conditions of temperature and it is this consideration which leads one to investigate the systems of drainage

In the four basic systems of drainage, one is promiscuous and the rest are designed for the welfare of the community In these systems the offending feature is the liability to retain collections of water Cement drains and (cement)? cess pools fall in one group and non-cement drains and absence of any system in another group as there is in the latter case a possibility for the water to evaporate or to permeate into the soil

The data may be reduced to four concrete questions

(1) Is there a direct correlation between the incidence of infection and a particular system of drainage?

The evidence may be looked for in the families and groups of the population of each area (Table I) In families, the evidence is direct (as the members reside under uniform conditions), in groups, it is indirect

The value of a single system of drainage in percentage figures is as the following —

C D, fam, 17, group, 17 9, N S, fam, 9 3, group, 9 2, N C D, fam, 9 5, group, ?, C P, fam, 5, group, 10

The evidence is convincing regarding cement drain and absence of any system

(2) What combination of systems influences the filarial situation in a dwelling?

The evidence of the cases in percentage figures is as follows —C D and R, 43 (fam) and 38 (group), C P and R, 17 (fam) and 15 7 (group), C D and C P, 14 3 (fam) and 37 5 (group) Figures in other combinations are small.

It appears that the presence of reservoirs act in a sense like a mordant and the system of cement drains whether singly or in combination, especially with cess pools, determines the *severity* of infection both in percentage and in signs

(3) Is there a definite order of intensity in individual areas on the lines of the results obtained?

It was not possible to study every area as closely as Bihar owing to questions of facility and time But wherever the evidence is discernible, it is noted that the system of cement drain tops the list

The effect of systems like cess pool and non-cement drain appear to be interchangeable Wherever there is a juxta-position of cement drain and cess pool, the value of cess pool is intensified

(4) Is the evidence supported by the signs of filariasis?

The answer is in the affirmative The situation is discussed under the heading C and Table III and the evidence is clearly brought out under the Bihar area

Significance of the evidence I have postulated (*vide* Introduction) that one of the correlated factors which govern an *intensive* endemic area is the presence of urban or suburban population Areas, like the above, usually are provided with the different systems of drainage The explanation of that assertion may now be looked for in the systems of drainage which freely allow *C fatigans* to breed, more especially cement drain and cess pool

Incidence in relation to occupation Once the cement drain is accepted as one of the prolific sources of breeding of *C fatigans* and *contributing largely* to human infection, it is obvious that the population residing on that system of drainage is exposed to the maximum infection in nature Traders usually occupy the front stalls in a thoroughfare and almost in all cases the main thoroughfare is the one place provided with the cement drain system Although the evidence is supported by the data given under Table III, to a certain extent, the infection in the trading class alone should be considered of secondary importance

The reason is that even well-to-do people who apparently live under good hygienic conditions are exposed to a considerable degree of infection, when they neglect to keep their *drainage system clean*, as is evidenced by the history of the family given under the Bihar area

V Conclusions

1 That, in an area whose physiographical and physical characters are like those of Bihar and Orissa, the evidence of infection by *F bancrofti* may be considered from the point of view of the degree of infection of the inmates of dwellings situated on different systems of drainage for the disposal of house water, the systems being cement drain, cess pool, non-cement drain, and absence of any system

2 That, among such systems, a cement drain will prove an offensive system from the filarial view-point, if the house water is allowed to accumulate, say for a period of 8 days

3 That families and sections of the population residing on the cement drain system show a higher percentage of infection than those residing on other systems

4 That in the area watered by reservoirs or artificial tanks the percentage of infection in the inmates of the dwellings situated on the different systems is proportionately and highly intensified, although there is no evidence to show that the invertebrate host is breeding in reservoirs or tanks

5 That a combination of drainage systems in an area is more dangerous than a single system

6 That a vicious combination appears to be that of cement drain with cess pool or non-cemented drain Cases showing incidence and signs of filariasis are of more frequent occurrence from the dwellings situated on such systems, especially the first two

7 That in the age and sex incidence the percentage of infection in adult males is 22, adolescent males, 10, adult females, 9 6, adolescent females, 7 5

8 That no class of population is exempt from infection if residing on the more injurious systems of drainage from the filarial stand-point

9 That *C fatigans* will breed in all the systems of drainage under winter and summer conditions

10 That, from the view-point of filarial prophylaxis, the evidence is concrete and the first step in prophylaxis is to keep the cement drain and cess pool systems in strict sanitary order

REFERENCES

Korke (1930*a*) Observations on the correlation between the incidence of filarial infection in the human host and in the insect carrier in relation to terrain Part V *Ind Jour Med Res*, XVIII, 1, p 319

Idem (1930*b*) Observations on the characters of filarial endemic areas in Bihar and Orissa Part VI *Ibid*, p 333

OBSERVATIONS ON EXPERIMENTS DESIGNED TO COMBAT DRACONTIASIS IN AN ENDEMIC AREA BY COL MORISON'S METHOD OF 'LIMING WELLS'

BY

RAO SAHEB Y M PRADHAN, M C P S (Bom)

[Received for publication, May 5, 1930]

AMONG the tropical diseases that cause the most intense and prolonged suffering and misery, guinea-worm disease (DRACONTIASIS) occupies a very high place

This disease is endemic all over the Bombay Presidency and manifests itself in seasonal epidemics Gujrat, Konkan and the Deccan are predominantly subject to its ravages Statistics reveal the fact that on an average 10 per cent of the population in the Colaba district is infested with guinea-worm disease during the epidemic season, i e, from February to May, the climax being in the month of March, which is notorious among those subject to the disease on this account

Taking the average duration of the disease to be three months, owing to negligence of the sores, the appalling waste of labour which the disease entails on the population can be judged from the fact that out of a total population of 1,000, there will be 100 people incapable of following their daily avocations during the period of guinea-worm prevalence The prolonged incapacity for work seriously adds to the gravity of the situation from an economic point of view It is not unknown, that in some cases, when the disease affects joints and vital parts of the body, it assumes a grave aspect and instances are not rare, when sufferers have been crippled for life and disabled from earning their livelihood This brings woeful calamity to the family, if the sufferer happens to be the bread winner From the statistics recently collected, it is worth while noting that affection of all joints reaches 23 3 per cent, that of the ankle being 80 per cent of the total joint affections (For percentages of seat of lesions *see* pp 448-449 and Graph 3) The high percentage of total joint affections, with 80 per cent involvement of the ankle joint, is appalling, it being the chief factor

responsible for ankylosis or stiff joints and other deformities resulting from the disease

Though the life history of the guinea-worm parasite has been successfully studied and confirmed long ago by many authorities such as Fedtschinko, Leiper, Castellani, Liston and others, yet, as has been rightly remarked by Hamilton Fairley, but little attention has so far been paid by the general public, or the medical profession, to the practical application of this knowledge towards inventing prophylactic measures against this scourge, for which curative measures have but a limited scope and success

Various treatments, local as well as general, have proved a miserable failure Even drastic measures like intravenous injections of varied preparations of arsenic used as the sheet anchor in treatment for *Treponema pallida* and other akin parasitic infections and of drugs like tartar emetic in 4 per cent solution (Macfie), together with subcutaneous injection of corrosive sublimate, 1 in 1,000 (Emily), and chinosol (Acton) have not had the desired effect

Injections of alcohol (Foulkes), opium and formalin into the body of the parasite, have also met with doubtful success Surgical treatment is the only and the best curative treatment, but it has its own limitations according to the stage of the disease and is generally shunned by the lay public in the mofussil

The ideal measure, therefore, for relieving suffering humanity lies in attempts directed towards prophylaxis and this can be achieved, as is universally acknowledged but indifferently adhered to, in two ways (1) prevention of contamination of water for human consumption, (2) destruction of the carriers of the disease, viz, *Cyclops* The first is dependent on (*a*) improvement of water sources and then proper protection by means of engineering devices, such as covering the draw-wells and providing these with Mayer's hand pump or force pump, and (*b*) by educative propaganda through lantern slide demonstrations, coloured and instructive posters, etc, etc

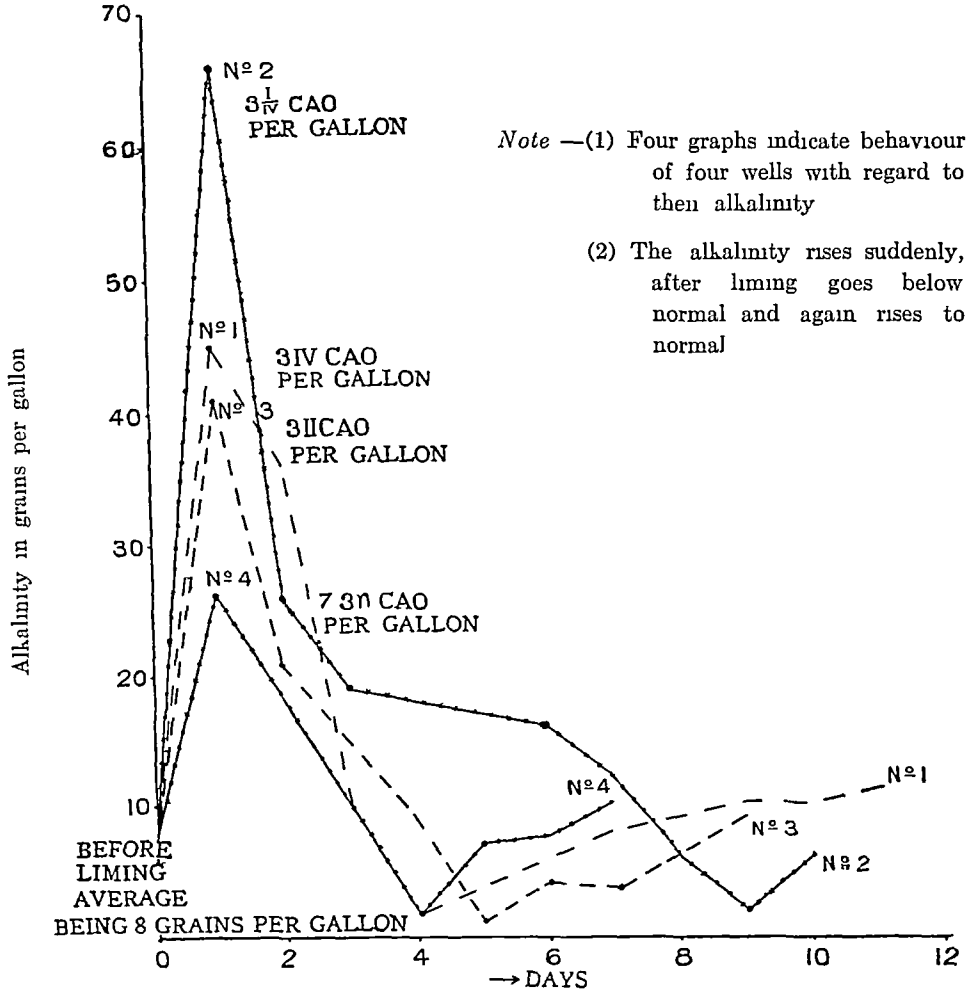
The first way, however, is dependent on the help and co-operation of the public and involves a serious monetary problem (*vide* estimates, Appendix V *a* and *b*) which the public or Government can ill-afford The second way, viz, destruction of *Cyclops*, would be a more ideal, effective and safer measure, if successfully achieved, as it can be carried out independent of the public and would be more economical

To meet this end, Col Morison carried out laboratory experiments with various drugs and observed that lime (CaO) was the cheapest and most effective

To utilize this cheap, simple and feasible finding, the Director of Public Health tapped the resources of the Indian Research Fund Association, who very kindly offered a generous grant of nearly Rs 30,000 to institute field experiments, to establish the value of 'lime disinfection' on a large scale, in the mofussil

Lines for experimentation and observation were outlined and the work was commenced from February 1928 After two months' touring through the district to select suitable villages for experiments, to study the geographical

GRAPH 1*a*



Graph showing alkalinity of water of four wells before and after liming

incidence of *Cyclops* and guinea-worm disease, as well as to repeat some preliminary laboratory and test experiments in the field, actual operations were started in May 1928

SUMMARY OF THE FINDINGS OF EXPERIMENTS AND OBSERVATIONS ON 'LIME TREATMENT' IN THE FIELD

The total number of wells in Panvel and villages within a 5 miles radius were 27,—15 being private property, 8 Municipal and 4 Local Board wells The

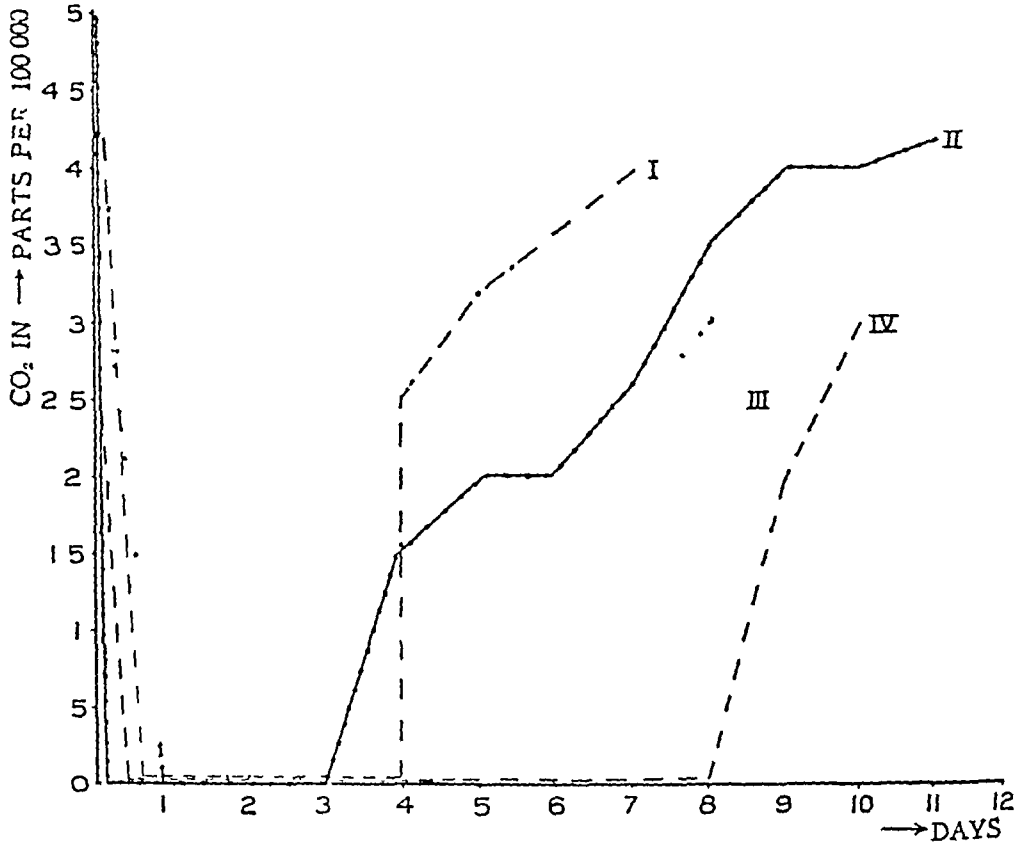
private wells were available only for one treatment but the Municipal and Local Board wells, numbering 12 only, could be subjected to repeated periodical treatment The following is a description of 66 total limings, including

GRAPH 1b

Graph showing behaviour of CO_2 before and after liming

Note—CO falls to zero immediately after liming and again rises to normal gradually in from 7 to 10 days

Note—For convenience of expression the fall is shown gradual although it is sudden in fact



machine' liming as well as 'hand' liming, done on 27 wells, from the last week of December 1928, till May ending 1929 —

Summary of 66 total limings

Number of limings	Dose of lime per gallon	Number of wells
53	One drachm	28
5	Two drachms	5
5	Four drachms	5
2	Half drachm	2
1	Three drachms	1

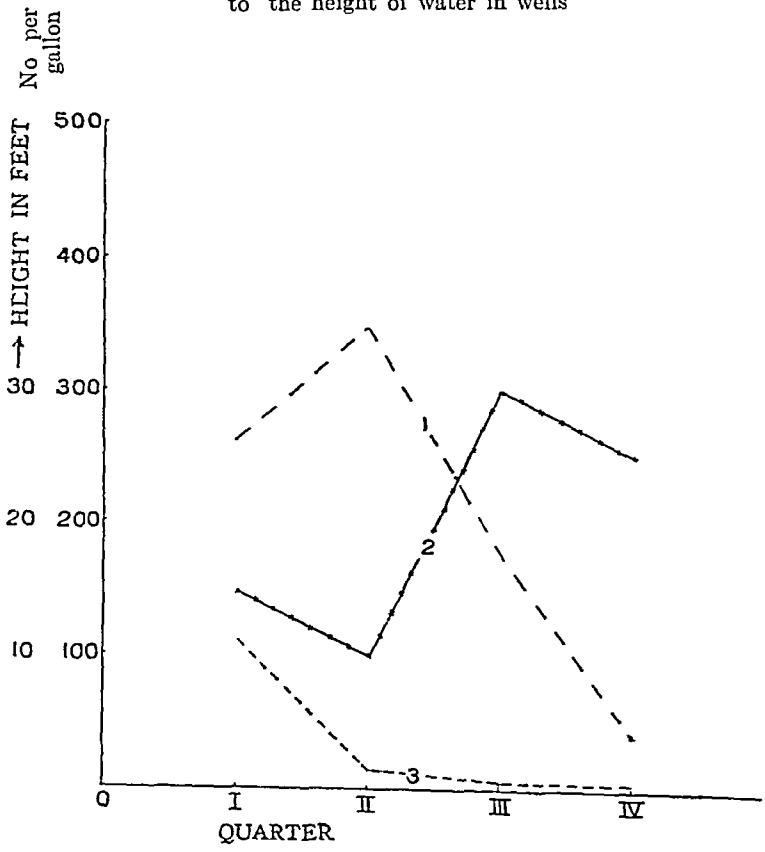
The summary and detailed tabulated schedules of liming treatment on the 12 wells selected for repeated periodical limings are given in Appendix I

Statistics of guinea-worm cases before and after the experiments, in the group of villages under experiment and those under control, distinctly prove the benefit derived in diminishing the 'case-incidence' in experimented villages, if not in reducing it wholesale (*vide* Appendix II)

Hopes can, therefore, be safely entertained for complete eradication of the disease in the course of time, if the liming propaganda is reinforced by the

GRAPH 2

Seasonal guinea-worm and *Cyclops* incidence in rotation to the height of water in wells



1 Represents *Cyclops* per gallon
2 Represents height of water in wells in feet
3 Represents guinea-worm incidence in number

provision of a perennial water supply and the hearty co-operation of the people, both being most important and inevitable adjuvants to any prophylactic propaganda against this scourge

Details of the processes of 'machine' and 'hand' liming and their respective advantages and disadvantages together with a rough estimate of their cost are given in Appendices IV and V (*a* and *b*) and in estimates given later in the body of this paper

GEOGRAPHICAL AND SEASONAL GUINEA-WORM INCIDENCE

The survey of 360 villages in 7 talukas and 2 pethas of the Kolaba district, instituted by the staff of the Assistant Director of Public Health, W R D, Nasik, in 1925-26, reveals the following figures of 'case-incidence' in each taluka and petha, in relation to their respective population (*vide* Appendix IV)

The average of 5 per cent 'case-incidence' is not reliable *in toto*, because although the survey lasted for nearly two years, every village or taluka could not possibly have been inspected during the epidemic season, as it should have been, to obtain fairly accurate data

To substantiate this, I may quote the statistics of one of the villages in Panvel taluka, taken personally by me with the aid of the Talati and the Circle Inspector, during the height of an epidemic

Statistics of village Kamotha (Taluka Panvel)

Population	295
Number of houses	60, infected 33, i e, 55 per cent
Persons infected	54, i e, 18 per cent on total population and 27 per cent on 204, the population of the infected houses

Sex infection

Males	50 per cent
Females	22 „ „
Children	28 „ „

Predilection of seat of lesion of 54 cases having 161 worms

Foot	45 per cent	85 per cent lower extremities
Leg	34 „ „	
Ankle	6 „ „	
Trunk and upper extremities	15 „ „	

The statistics of four villages under experiment and observation taken in March 1928 prior to starting experiments are also interesting and are, as given below —

Population of four villages	2,010
Number of persons infected	120, i e, 5 8 per cent
Number of worms in 120 cases	150

Sex infection

Males	47 5 per cent	
Females	25 „ „	
Children	27 5 „ „	

Predilection of seat of lesion of 150 worms

Foot	33 per cent	Lower extremities 92 per cent (*vide* Graph 3)
Leg	32 „ „	
Ankle	19 „ „	
Knee	5 „ „	
Thigh	3 „ „	
Trunk and upper extremities	8 „ „	
Total joint affection	23 „ „	
Ankle involvement in the total joint affection	80 „ „	

It is the affection of joints which is chiefly responsible for lifelong incapacitation of the victims as already stated Rich or poor, young or old, male or female, all are equally susceptible, some people suffer regularly every year, while others escape as a rule, being perhaps immune or non-susceptible to the disease The disease does not exempt caste or creed

The maximum number of guinea-worm cases were obtained in the first quarter of the year, the highest being in the month of March In the second quarter, the number appreciably dropped, the total number observed being 13 only, while in the third quarter the number was noted to be insignificant (*vide* Graph 2)

A few sporadic cases occurred throughout the year, though it was not uncommon to meet with a few epidemic outbreaks, even during the monsoon, as in the villages Kamotha and Kolkha

GEOGRAPHICAL AND SEASONAL INCIDENCE OF *Cyclops*

Over five hundred samples of well waters examined revealed the presence of *Cyclops* in cent per cent number A hundred-and-sixty samples of well waters, obtained by the courtesy of Mamlatdar Mr Katti, through his village officials for studying 'Average Alkalinity and Free CO_2,' revealed only 17 per cent of 'Well Infection,' which differs strikingly from the cent per cent results above alluded to and obtained from the samples collected personally during touring This is probably explained by the facts that the village officials were ignorant of the habits of the crustacea met with, these having a tendency to be at the bottom of the wells (bottom feeders) and that their samples were probably taken from near the surface

Of the four quarters of the year, *Cyclops* were found to preponderate in the second one, being most marked in number in the month of May (possibly owing to concentration of volume of water) and least at the end of October, the average number of *Cyclops* per gallon of water in each quarter being as under (*vide* Graph 2) —

Quarter and months	Number of *Cyclops* per gallon	Volume of water in height
1st quarter (Jan to March)	265	10 to 15 feet
2nd quarter (April to June)	350	0 to 10 „
3rd quarter (July to Sept)	175	25 to 30 „
4th quarter (Oct to Dec)	39	15 to 25 „

They declined in number to an appreciable degree in October, and multiplying again from November, reached their climax in the month of May (*vide* Graph 2) It is not proposed to suggest that these data regarding prevalence of *Cyclops* are in any way definite, as the observations were confined to a limited number of wells and the examination of water from different places was done on different dates of the month, the volume of water at each examination, which governs this calculation, was also not scrupulously respected

Local species of Cyclops—Only two species of these Copepoda were observed, viz —

(*a*) one with long antennæ and antennules, and

(*b*) one with short antennæ and antennules

Species (*b*) was observed in experiments, *in vitro,* to be capable of infection with guinea-worm embryos, added from without, within two or three hours, while the species (*a*) was not infected for days together Both species were found to remain alive in the laboratory for over a month

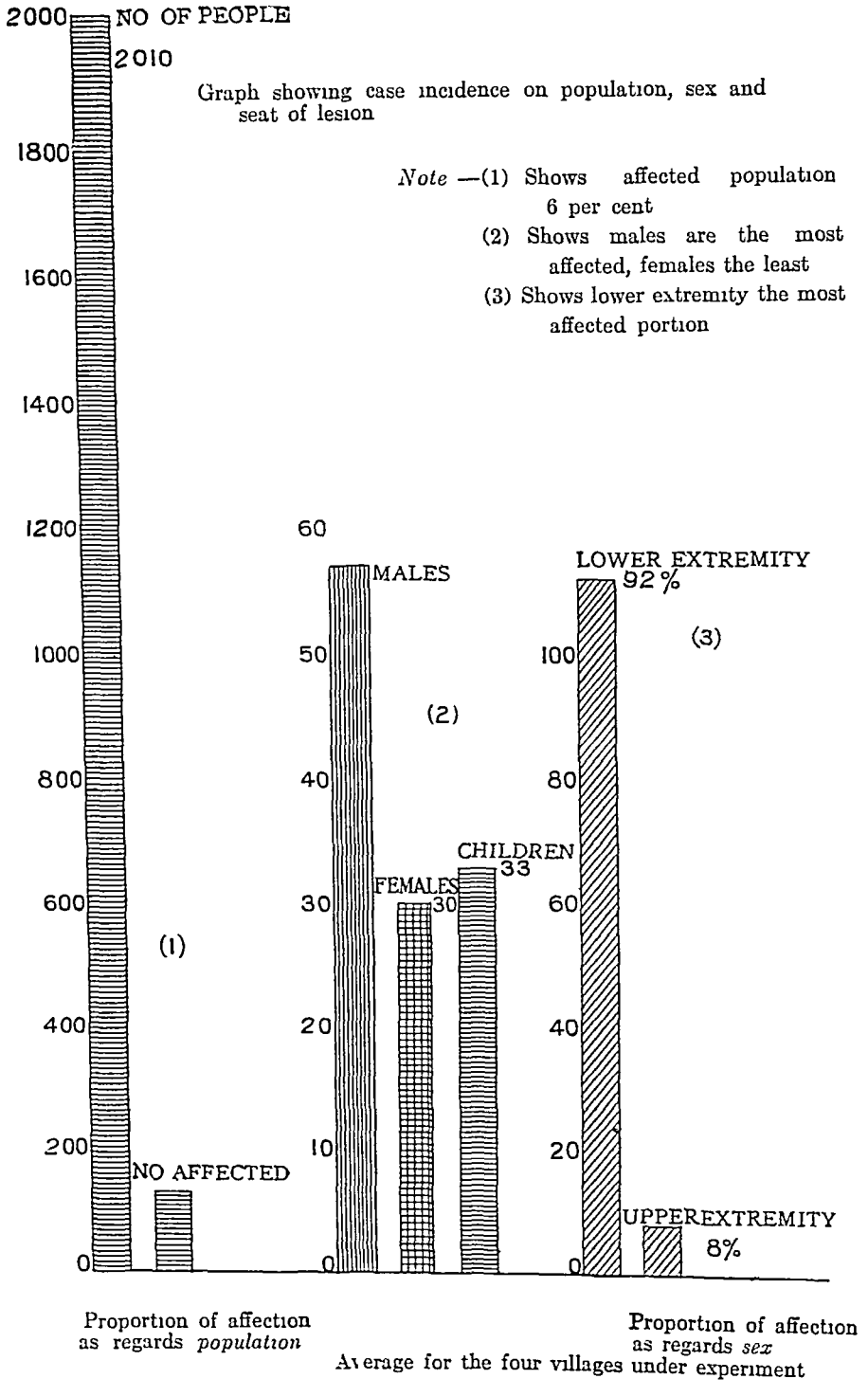
Whereas doses of CaO as large as four drachms per gallon, in field experiments, failed to prevent regeneration of *Cyclops* from eggs, doses as small as ten grains were found to be effective in laboratory jars, as no eggs hatched out in them for nearly two months

The nauplius (larvæ of *Cyclops*) hatched out from the egg when kept under observation, was seen to develop into an adult crustacean in from six to eight days and the intermediate developmental stages were roughly observed It would be interesting and important to study the bionomics of this crustacean in connection with the prophylactic campaign involving their eradication

Fish problem—A couple of wells in which a large number of fish were swimming and darting were found to be entirely free from *Cyclops* This naturally gave an impetus to find out if fish might be responsible for absence of *Cyclops* in wells We were able, by obtaining fish from wells through the Mamlatdar of Pen, to subject them to experiments

The following were the fish obtained from wells and various sweet water collections during the monsoon and found to feed on *Cyclops,* in different degrees, at different ages Some of them are proved mosquito larvæ eaters

GRAPH 3



Graph showing case incidence on population, sex and seat of lesion

Note —(1) Shows affected population 6 per cent
(2) Shows males are the most affected, females the least
(3) Shows lower extremity the most affected portion

Proportion of affection as regards *population*

Proportion of affection as regards *sex*

Average for the four villages under experiment

(1) *Rasbora daniconia*—Feeds on small crustacea and mosquito larvæ, though described as vegetarian by some, eats larvæ in early life

(2) *Haplochilus lineatus panchax*—Surface feeder, eats *Cyclops* if driven to do so by absence of other food

(3) *Barbus phutunio*—Occurs in shallow collections of water, eats *Cyclops* voraciously

(4) *Polycanthus cupanus*—Can also live in brackish water (Liston), eats *Cyclops* indifferently

(5) *Nemochilus*—Bottom feeder, a moderate *Cyclops* eater

The identification and bionomics of these fish have been roughly ascertained by my friend Mr D D Sivartha, B Sc, Assistant to Professor of Zoology, Fergusson College, Poona, to whom I am greatly indebted for the kind help voluntarily advanced

The above findings suggest an independent and very important problem, opening a new field for investigation, especially in connection with ponds, which, at places, form the only and common source for animal as well as human consumption and which are responsible for the disease being obstinately endemic in such places

It is needless to say that if breeding such fish in various water sources be successful in *Cyclops* destruction, eradication of the guinea-worm disease would be easily achieved, and all the elaborate, technical and costly propaganda for the purpose done away with

PRELIMINARY EXPERIMENTS TO DETERMINE EFFECTIVE DOSE OF CaO

Fourteen test-tubes were taken and to each was added —

(*i*) 28 c c (1 oz) filtered and *Cyclops*-free water, having 9 5 grains of alkalinity per gallon and 3 2 parts of free CO_2 per 100,000 parts,

(*ii*) lime (with 80 per cent available CaO) in doses varying from ¼ to 12 grains,

(*iii*) five adult *Cyclops*

Tubes were vigorously shaken and were allowed to stand, the result being closely watched The *Cyclops* were found dead in all the tubes within half an hour, but as practically all the lime in suspension was precipitated within 15 minutes and since the *Cyclops* have a tendency to be at the bottom of the vessel, it was presumed that they were mechanically buried under precipitated lime and were killed by pressure and suffocation

The test-tube experiments were therefore abandoned and replaced by 'Jar Experiments' This, in addition to removing the aforesaid drawbacks and other discrepancies, facilitated the experiments, which could be done in a greater volume of water (1 pint) to simulate roughly the phenomenon in nature and afford clear observation

Results—The *Cyclops* in the jar containing 10 grains of CaO (80 per cent strength) were found dead in ten minutes, those in the jar containing 5 grains died within 20 minutes, while those in the jars containing 3 and 4 grains of CaO were observed to have died within half an hour In the

jar containing 1 and 2 grains *Cyclops* remained alive for 40 minutes and in the last jar containing ½ grain only, they survived for three hours

The experiments repeated with CaO of 66 per cent strength took comparatively longer time with respective doses, the minimum period with the maximum dose being 15 minutes as opposed to 10 minutes with 10 grains CaO in the former experiment

Experiments for testing effects of CaO on other Crustacea, etc—Similar experiments were tried on the nauplius (*Cyclops* larvæ) and other water animals, viz, *Paramœceum*, *Daphnæ* and larvæ of mosquitoes and flies, which all perished with 10 grains dose of CaO

Experiments for testing effects of CaO on eggs of Cyclops—One jar containing eggs of *Cyclops* only and treated with 10 grains of CaO was kept under observation No embryos were found developed for over a couple of months

Experiments for studying the period of development of Cyclops *from eggs*—A jar containing well-water with the mud and deposit and impregnated *Cyclops* was kept under observation After the eggs were dropped, the *Cyclops* were filtered off and fresh water free from *Cyclops* was added to make up the quantity During a week's time, nauplius and adult *Cyclops* were seen in the jar This gives an approximate period of development of this crustacean from eggs but needs be confirmed by further experiments as it has a direct bearing on the frequency with which limings should be repeated

Alkalinity and free CO_2—These were estimated for each individual well before liming to regulate the dose of CaO, and observed every day subsequently for a week or ten days, until the well again regained its normal point

It may be observed from Graph 1*a* that the alkalinity of water treated rose immediately after liming to about 4 or 5 times its original amount, dropped by about 8 to 10 grains per gallon for the first four days, subsequently decreased gradually and eventually going below the original point, came up to normal within a week or ten days

The free CO_2 disappeared immediately after liming It made its reappearance on or about the 4th day and steadily rose to its original value by the beginning of second week (*vide* Graph 1*b*)

Average alkalinity and free CO_2—As the alkalinity and free CO_2 of the water to be treated influenced the optimum dose of lime, their respective average strength was determined, to facilitate ready calculations, by examining 160 samples of water, obtained by the courtesy of the Mamlatdar of Panvel The maximal alkalinity and free CO_2 were 31 5 grains per gallon and 25 0 parts per 100,000, while the minimal were 1 0 grain per gallon and 0 5 parts per 100,000, the average being 4 8 grains per gallon and 3 2 parts per 100,000, respectively These value though immaterial for two drachms dosage of CaO may have an important bearing in modifying the dose, if a larger one were required to be used to ensure destruction of eggs of the *Cyclops*

Average strength of lime (for available CaO) —The question of estimating the strength of lime available on the market is of prime importance, as it has a direct bearing on the modification of the constant dose determined

It cannot be overlooked as there is a wide margin of difference in the strength of any two given samples of lime and it is subject to deterioration in quality by exposure if not properly tinned and carefully preserved

A number of samples examined by titration against N/28 sulphuric acid solution gave an average strength of available CaO ranging from 60 to 80 per cent The dose was therefore accordingly modified by increasing or decreasing the amount of CaO used, as necessary

Fitness of water after liming with different doses of CaO and regeneration of Cyclops —The following is the description of 66 limings, including 'machine' as well as 'hand' liming, done on 27 wells from the last week of December 1928, till May ending 1929, with different doses —

Dose of CaO in drachms per gallon	When water was potable	When *Cyclops* regenerated
One	3rd day	Two weeks
Two	4th day	Three weeks
Three	5th day	Four weeks
Four	7th day	Six weeks

The liming was started with four drachms dose of CaO to a gallon of water to begin with, the dose having been calculated by doubling the laboratory dose and allowing for alkalinity and free CO_2, as well as the strength of lime used in respect to available CaO therein

The dose was found most effective in killing the *Cyclops* instantaneously, the period for reappearance of the crustacean being over six weeks The water, however, was found to be unfit for drinking for nearly a week Attempts were, therefore, made to ascertain the smallest optimum dose in order to curtail the period of unfitness of water for drinking, since this factor seemed to be of paramount importance, owing to great scarcity of water in this district, especially in the hot season

The dose was gradually reduced to one drachm per gallon and was found to be equally effective in its immediate results, with the advantage that the water was fit for drinking after two days only, although the period for re-appearance of *Cyclops* was, as compared with the results of four drachms dose, much shorter, i e , two weeks Further reduction to half a drachm dose, having proved ineffective, was discarded

Attention was, therefore, centred on the effects of one drachm dose, with which fifty-three limings were done up to the end of May, the remaining

thirteen having been done as shown in the table given in the first section of this paper The smallest optimum dose thus determined was one drachm of CaO (80 per cent available lime) per gallon of water, four limings with which, at periodical intervals, before the probable period of re-hatching of eggs, has been observed to keep the wells free from *Cyclops* over two months

Successive limings with the said dose prolonged the period of regeneration of *Cyclops* by one to two weeks each time and some of the wells under experiment, treated at due intervals (i e , before the reappearance of *Cyclops* after each liming), have kept free of *Cyclops*

Since, even a four drachms dose of CaO fails to be effective on the eggs of the crustacean, their regeneration is inevitable, the period depending upon the dose used

Whereas a four drachms dose—the maximum used so far—has the advantage of prolonging the period of regeneration of *Cyclops*, it has a serious disadvantage of not allowing the water to be fit for drinking for nearly a week which the people can ill-afford to wait for, owing to great scarcity of water and the limited number of wells in the villages of this district

Chemical analysis of water limed with different doses of CaO—The Haffkine Institute, Parel, advanced the courtesy of examining samples of water limed with different doses, sent to them and the results were as under —

	Total solids	Temporary hardness	Permanent hardness
Shringarpure's well before liming	56 8	25 0	1 0
Shringapure's well after liming	133·8	72·2	48·0
Durga well after liming	47 4	7 0	Nil
Virupax well before liming	66·8	32 6	4 4
Virupax well after liming	43·6	8·6	4 4
Square well after liming	115 2	32 2	4 8

N.B—All results are expressed in parts per 100,000 The analysis was kindly done at the Haffkine Institute, Bombay by favour of the Director, Major L A P Anderson, I M.S

DESCRIPTION AND METHOD OF USE OF THE 'BAICO' FIRE-FIGHTING MACHINE

The 'Baico' machine (*vide* Plate XXIX), which has one suction and two delivery pipes, is connected to a galvanized tank of the capacity of 70 gallons by means of one of the delivery canvas pipes from the engine, the other being used as a safety outlet, letting out excess water, to prevent overflow from the tank

The suction power of the engine being 200 gallons per minute, if the engine is worked under 20 to 30 degrees combined vacuum pressure, the tank, having a capacity of 70 gallons, overflows

The speed of the engine has, therefore, to be regulated to prevent overflow and the engine working at this rate occupies forty seconds in pumping out 70 gallons

A gallon of petrol is consumed for each hour of the working of the machine and the maximum time for working in the 1st quarter of the year did not exceed 2 hours

Suction of the water from the well, its mixing with the lime in the tank and the delivery of the lime solution back into the well, through a delivery pipe from the tank, take place simultaneously A tin of lime, i e, 35 lb, held in a perforated drum, which is pivoted on to the tank, takes about half an hour to completely dissolve The maximum quantity of lime used for an average well is 2 to 3 tins in this season

The time for working the engine on a given well is chiefly governed by the quantity of water, which has to be circulated through the engine and the tank at least once

The thorough churning of lime and the speed with which the work is carried out are the obvious advantages which the 'Baico' can claim whenever and wherever it is possible to make use of it

Method of 'hand' liming

The process of 'hand' liming is very simple and mainly differs from that of 'machine' liming, in evacuating the well by manual labour

The same galvanized iron tank with a perforated drum suspended in it is made use of and the water is drawn by two gangmen by means of buckets This being the case, it is but natural that it occupies a little more time than machine liming Although the churning is not so thorough as in machine liming, the ultimate result is practically the same, with a decided advantage in cost and utility in the interior, where the 'Baico' apparatus cannot be conveniently carried

It takes about 10 to 15 minutes to fill the tank by means of two buckets, each of 2 gallons capacity The estimated dose of lime is then put into the perforated drum of 5 cubic feet capacity in 2 or 3 instalments

The drum is rolled by two gangmen and the water is replenished from time to time letting out the lime solution into the well The rotations of the drum are from 15 to 20 per minute The operation takes about 40 minutes to dissolve one tin of lime, i e, 35 lb On an average, two tins are required for a well of 12 feet diameter with 15 feet height of water, i e, containing a total volume of 10,697 gallons of water, in the 1st quarter

From start to finish, the operation takes roughly a couple of hours, including adjustment of the apparatus, etc, if the well is within 100 yards from the main road

COMPARISON OF RESULTS OF 'MACHINE' AND 'HAND' LIMING

Advantages of the 'Baico' apparatus —

(1) Quicker and greater amount of work
(2) Thorough churning of lime
(3) Very useful for work on a large scale

Disadvantages —

(1) Very expensive
(2) Needs a mechanic to work it
(3) Needs a garage
(4) Needs a battery to work the Ford engine
(5) Needs a lorry or a bullock cart to drag it
(6) Petrol consumption, wear and tear of tubes, tyres, etc
(7) Inability to take it into the interior and up the hills
(8) Inability of 'Baico' to suck up water below 25 feet depth

Advantages of 'hand' liming —

(1) Almost equal efficiency
(2) Utility of application in the interior and in the hills where 'Baico' cannot be taken
(3) Very economical
(4) Water becomes fit for drinking earlier, owing to earlier precipitation of lime
(5) Apparatus required is cheap and simple and portable
(6) No recurring or wear and tear expenses
(7) Saving of initial cost of elaborate machinery and technical staff required to work it
(8) No oil, petrol and garage expenses

Disadvantages —

(1) Tedious process
(2) Requires manual labour for pumping out water
(3) Want of thorough churning of lime (this is immaterial, as the results are not appreciably affected)

Only three advantages against eight disadvantages of 'machine' liming as compared to diametrically opposite ratio in the case of 'hand' liming prove the distinct inferiority and undesirability of the former, especially for this district

TREATMENT WITH POTASSIUM PERMANGANATE

At the instance of Col F P Mackie, CIE, IMS, Officiating Sanitary Commissioner with the Government of India, and Secretary, Scientific Advisory Board, Simla, a few experiments with potassium permanganate were carried out with the following result —

The dose used was two ounces for 1,000 gallons of water being double of that used for destroying cholera vibrios *Cyclops* were found to have been

alive for twenty-four hours This, together with the fact that the water remains tinged for nearly a week and is unfit for drinking, proves the unsuitability of this drug for the purpose

Chief causes for endemicity of disease in this district

The chief factors responsible for the propagation and perpetuation of the disease for years in this district are as follow, in order of their importance —

(*i*) Impure and inadequate water supply
(*ii*) Dilapidated condition of wells
(*iii*) Ignorance and dirty habits of the people

The small percentage of Local Board wells in proportion to the population, and the total water sources in the district with only 50 per cent of them providing perennial supply of water, sufficiently testifies to the inadequacy of the water provision The number of Local Board wells, in need of repairs which, in some of the talukas like Mangaon, may form as high a percentage as 25 per cent, is too great to be ignored not to speak of the private wells of which as many as 44 per cent may be in need of repairs

As far as guinea-worm disease is concerned, an entire absence of parapets, or the presence of defective or broken ones, are fruitful factors leading to pollution and contamination of water, since such allow the spilt water to run back into the well

Furthermore, want of the cemented plinths around the wells and absence of or defective cementing of the wells from within, allow subsoil water to percolate through and contaminate the well water Thus, the importance of the construction and improvement of water sources cannot be too much emphasized

Water problem —Sufficiency of drinking water is a primary need for human existence The problem of pure and sufficient water supply is of paramount importance, as nothing has a more direct and vital influence on the health and physical well-being of people in tropical countries

Nothing impressed me more painfully in my tour in the interior of some of the talukas of the district, than the scarcity of drinking water In a good many places, people were found drinking muddy water, which was unfit for consumption even by cattle In some places, the very appearance of the water was repulsive and a stinking smell actually emanated from it This being the only water source, people had no alternative but to drink it It is an ordeal which the rural populace has to undergo during every hot season owing to drought Some of the waters, if it will not be thought an exaggeration, were exactly as Dr Bentley's assistant rightly described them 'Poisonous solution of foul organic impurity and a liquid misnamed water' Thus it is evident that the scarcity of water in this district in the hot season is one of the most potent factors responsible for the endemicity of guinea-worm disease

Colaba district is formed by the narrow strip of land about 20 miles in breadth, lying between the Western Ghats and the sea It is bounded on the North by Thana district and on the South by Satara and Ratnagiri districts, the Ghats and the sea being roughly the Eastern and Western boundaries

The average rainfall is 100 inches and only in exceptional years is it below the normal The rainy season usually begins with June and ends with October, July and August being the wettest months The district is mostly hilly and is intercepted by creeks It may seem curious that in spite of the heavy downpour of rains, there should be an acute shortage of drinking water throughout the whole district, especially during the hot season, from March to May

Owing to its close proximity to the sea, the rain water immediately drains off into the sea There are no rivers to speak of Towns and villages are mostly dependent on well water The subsoil water is probably scanty and with some exceptions, it is not available in April and May Most of the district appears to have a hard trap rock about six or seven feet below the surface, thus minimizing the possibility of plentiful underground water The soil is not retentive, being composed of sand and clay and the substratum of hard murum and rocks causes the water to drain rapidly into the sea

Brief summary of conclusions

1 The efficacy of lime treatment for *Cyclops* destruction is undeniable

2 Though the minimum optimum dose of one drachm may need 4 to 6 repetitions of limings during the season, a larger dose (*immediately neutralized with* CO_2) may be effective in destroying eggs and preventing regeneration of *Cyclops*

3 Experiments concentrated on a limited number of wells have a decided advantage over those conducted on a large number

4 As there are only one or two Local Board wells in a village, it was not possible as a rule to do more than one well at a time

5 That 'hand' liming, besides being as efficacious as 'machine' liming, is far cheaper and more practicable in the interior and up in the hills Some heavily infested villages in this district are situated right in the interior and up the hills where the 'Baico' apparatus cannot go

6 The 'machine' liming, where practicable, would be desirable for operations on a large scale, but the cost would be great The Colaba district is unfit for machine operations owing to heavy rains and the awkward situation of the wells (in fields)

7 A large number of villages have ponds which are also used as sources of drinking water when wells dry up In some villages ponds are the only source for water supply, while in others there are neither wells nor ponds, dowras and temporary pits being the only sources available

8 Potassium permanganate treatment is inefficacious

9 Stringent measures against infection of wells, together with sufficient water supply and educative propaganda amongst the rural as well as urban population, are essential adjuvants of the scheme for its ultimate success

10 Since guinea-worm disease is seasonal, liming operations need only be done from February to the end of May, to prevent contamination of water by acute cases, so that it would be very economical

All above-mentioned findings need further extensive observations for their final confirmation and the fish problem should receive earnest attention of the authorities as it may not only solve the pond question but may perhaps replace other more elaborate or expensive propaganda

Before concluding I have to express my deep sense of gratitude to Col F P Mackie, C I I , I M S , for carefully scrutinizing my work at the time of his inspection as Officiating Sanitary Commissioner with the Government of India, and making valuable suggestions for trying the 'hand' liming process and the effects of potassium permanganate, as also for granting three months' extension for the work, in spite of great financial stringency in the budget of the Indian Research Fund Association I equally owe thanks to Col Morison, I M S , for having placed confidence in me to work out his scheme practically in the field My thanks are also due to the Director of Public Health for his invaluable suggestions from time to time I owe no less to the Director, Haffkine Institute, Parel, Mr Kikeri, the S D O, P W D, Colaba, Assistant Director of Public Health, W R D, Nasik, Assistant Professor D D Samarth and Messrs G R Shirgaipure, D M Gupte and the Mamlatdar of Panvel with his staff for their invaluable help and co-operation in their respective spheres

My special thanks are due to Mr Mandlick, Ex-Sheriff of Bombay, who very kindly permitted me to carry out experiments in his Inam village and spontaneously offered every assistance needed

Appendix I

Details of 12 wells under experiment treated with one drachm of CaO per gallon

	Title of wells	How often limed	When last limed	Presence or absence of *Cyclops*	Presence of *Cyclops* after each liming				
					1st	2nd	3rd	4th	5th
'Machine' limings	Vovle No I	4 times	27-3-29	Present	Present	Absent	Present	Present	
	Do No II	3 times	14-3-29	Present	Present	Present	Present		
	Kundevahal No I	4 times	8-4-29	Absent	Present	Present	Present	Present	
	Do No II	5 times	27-4-29	Present	Present	Absent	Present	Present	Absent
	Round well	3 times	13-4-29	Absent	Absent	Absent	Absent		
	Square well	Once (4 drachms dose)	12-2-29	Absent	Absent				
	Vadhala well	3 times	12-3-29	Absent	Absent	Absent	Absent		
	Cemetery well	2 times	25-2-29	Absent	Absent	Absent			
'Hand' limings	Vilupax well	4 times	20-4-29	Absent	Present	Present	Present	Absent	
	Krishnala well	3 times	5-3-29	Absent	Absent	Absent	Absent		
	Vovle No III	3 times	6-3-29	Present	Present	Present	Present		
	Kolkha well	3 times	23-2-29	Present	Present	Present	Present		

In 5 wells (4 'machine' and 1 'hand' liming) *Cyclops* disappeared after the first liming, as they were done at regular intervals

The remaining two wells have shown *Cyclops* throughout They were heavily infested with *Cyclops* and not 'pacca-built' They could not be treated the 4th time as they dried up

One out of 5 wells limed four times with 'hand' liming has proved sterile so far, the last liming having been done on 29-4-29 The remaining four showed regeneration of *Cyclops* after each liming as they could not be treated at regular intervals

Appendix II

Incidence of guinea-worm cases before and after experiments

		Before experiment, 1928			After experiment, 1929			Remarks
		March	April	Total	March	April	Total	
Villages under experiment	Voyle	52	11	63	20	6	26	55·5 per cent reduction
	Kundevihal	31	2	33	Nil	26	26	21·2 per cent reduction
Villages kept as control	Pendhai	12	Nil	12	7	8	15	
	Kalomboli	23	1	24	16	10	26	

Appendix III

Statement showing geographical incidence of guinea-worm cases per taluka in villages inspected

Number	Name of talukas	Number of villages in which guinea-worm survey was made	Population of the villages	Number of persons suffering from guinea-worm disease	Percentage of persons suffering from the disease on the population inspected
1	2	3	4	5	6
1	Mahad	90	18,972	3,008	6
2	Panvel	31	15,002	447	2
3	Roha	61	24,363	1,003	4
4	Mangaon	57	24,354	932	3
5	Uran Petha	25	18,372	1,451	7
6	Khalapur	13	3,954	203	5
7	Alibag	11	3,465	121	3
8	Karjat	4	928	60	7
9	Pen	68	28,319	2,126	9
	Total	360	167,729	9,350	46

APPENDIX IV

Comparison of 'hand' and 'machine' liming Cost of the material and staff

Expenditure on 'machine' liming	Rs	As	P	*Expenditure on 'hand' liming*
Petrol consumption for 'Baico' for one well for one hour, i e, for two hours, two gallons at Re 1-4 per gallon	2	8	0	
Do for Vanette to take the 'Baico' to the scene of operation	0	10	0	
TOTAL	3	2	0	Nil
Oil, wear and tear of tubes and other accessories for both	1	6	0	Nil
Establishment				
Pay of mechanic per day at Rs 3				Re 1-8-0 for two gangmen for each liming at the rate of As 12 each
Pay of cleaner per day at Re 1				
Pay of two gangmen each per day at As 12				
TOTAL	10	0	0	
For one well for one treatment	10	0	0	Rs 9-0-0 for six limings at Re 1-8-0 for each liming
For 6 limings for the season as under	60	0	0	
2 limings in February, 2 in March, 1 in April and 1 in May				
Cost of lime for each liming requiring two tins at Re 1-4 each	2	8	0	Rs 15-0-0 chunam charges for 6 limings at Rs 2-8-0 for 2 tins at Re 1-4-0 per tin
For 6 limings	15	0	0	
TOTAL	75	0	0	Rs 24-0-0

'Machine' liming				*'Hand' liming*
Cost of Ford Vanette, with its accessories	3,000	0	0	
Cost of 'Baico' apparatus	4,141	0	0	Nil
Insurance charges for the unit	150	0	0	
Galvanized tank with canvas delivery pipes and their wear and tear	260	0	0	Rs 260
Two delivery pipes and one connecting hose complete with coupling, etc	210	0	0	Rs 210
TOTAL	7,761	0	0	Rs 470

APPENDIX Va

Estimate for constructing a well and fixing pumps for a village having population of 1,000

(Reference P W D Handbook)

For constructing a well

	Rs
Cost of constructing a well 12 feet diameter having stone masonry including excavation in earth, hard murum and boulders, rock, paving outside, etc	1,400

For fixing up pumps

	Rs
Covering the same with reinforced concrete cover slab over rolled steel joints, etc, complete including providing a manhole and covering the same with cast iron covers	270

For a village having a population of 1,000 and taking the water consumption at 12 gallons per head the total amount of water required would be 12,000 gallons per day One Mayer's pump can pump out (600 × 8 = 1,800) per day taking that the pump will be working for 8 hours in a day

Number of pumps required would therefore be $\frac{120}{48} = 3$

Cost of providing and fixing Mayer's double acting force pump at Rs 100 each	300
TOTAL	1,970
Providing platform for keeping vessels and fixing pipe line as required, etc, complete	40
GRAND TOTAL	2,010

Note—Pumps go out of order very often and have to be repaired every now and then and hence the annual recurring expenditure may be taken at Rs 75 per annum

APPENDIX Vb

A more elaborate and expensive water supply scheme for a fairly big village or a small town *Population* 2,500 (Reference P W D Handbook)

Population 2,500

	Rs
Cost of constructing and covering the well is the same as before, i e, (Rs 1,400 + Rs 270)	= 1,670
As before, taking 12 gallons as the consumption per head, the total quantity required would be 30,000 gallons per day	
B H P required $= \frac{30{,}000 \times 10 \times 55}{33{,}000 \times 480} = \frac{25}{24}$, i e a pumping plant of 2 horse power will be required	
Initial cost of the installation	3,000
Cost of constructing an engine house	1,500
Cost of constructing a water tank of capacity 12,000 gallons in reinforced concrete	2,000
TOTAL	8,170
Working expenses at Rs 6 per day including pay of driver and lubrication oil, etc, complete, per year	2,160
Depreciation, repairs and interest on capital at 10½ per cent of the cost of the plant	315
Depreciation on masonry works and interest on the capital outlay at Rs 8	280
ANNUAL TOTAL EXPENDITURE	2,755

Note—In such installation it is advisable and also necessary to have a stand-by pumping set capable of doing the same amount of work for emergency

Explanation of Plate XXIX

Description of the disinfecting 'Baico' apparatus during operation on a well —

A 'Baico' apparatus
B Ford Vanette Tractor
C Galvanized Tank
D Perforated drum suspended in the Tank
E Suction pipe of the 'Baico'
F Connecting pipe to the Tank
G Delivery pipes from the Tank
H Well

A
B
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F
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G

THE ACTION OF A SYMPATHOMIMETIC ALKALOID IN *SIDA CORDIFOLIA* (*BRELA*)

BY

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HISTORICAL AND GENERAL

Sida cordifolia or '*Bala*' is considered to be one of the most valuable drugs in the Ayurvedic or Hindu medicine and has been largely used by the Hindu physicians from very ancient times. In the Mohammedan medicine it was used for its aphrodisiac effects. As no work on scientific lines has been done on this drug we made a systematic study of its chemical composition and medicinal properties.

The genus Sida belongs to the natural order *Malvaceæ* and plants belonging to this group are known in Sanskrit by the general name '*Bala*'. There are some seven or eight species but the Sanskrit writers make mention of five species of 'Bala' under the name '*Pancha Bala*'.

1. Bala—*Sida cordifolia* (Linn. H. F. B. I., 324, Roxb. 517) 'Brela' or 'Swet Berela'.
2. Mahabalâ—*Sida rhombifolia* Syn. *Sida orientalis Rhombodia* (Roxb. H. F. B. I., 324, Roxb. 517).
3. Nagbala—*Sida spinosa*, Linn. Syn. *S. alba*, *S. almifolia* Roxb. 516, 'Pila' or 'Peet Berela,' 'Bon Methi' (Beng.).

4 Atibalâ—*Sida rhombifolia*, Linn H F B I, I, 323, Roxb 517, 'Lal Barila' or 'Berela'

5 Rajbala—*Sida phaniphika, Sida capmifolia*, Linn H F B I, 323, Syn *S acuta, S lanceolata*, Roxb 517, Vern 'Pila' or 'Pit Berela'

There is another species known to the Sanskrit writers called 'Bhumibala' —*Sida humilis*, Willd H F B I, I 322, Roxb 516, or *Sida veronicifolia*

Of these species, the first one, viz, *Sida cordifolia* or '*Swet berela*' is the subject of our present investigation

Sida cordifolia, Linn (also known as *S herbacea*, Micans and *Rotundifolia*, Cav, *S althæifolia*, Swartz), known in English as country mallow, is a small herb which grows throughout the plains of India where the climate is damp It is known by the name of '*Bala,*' '*Batyalaka*' in Sanskrit, '*Kungyee* of *Khareti* or Barial' in Hindi, and '*Berela* or *Barila,*' '*Bala* or *Swet Berela*' in Bengalee The seeds are called *Beejbund*

It is distributed throughout tropical and sub-tropical India and Ceylon growing wild along the roadside in the villages It is a perennial undershrub with long branches, rooting at the nodes with scattered stellate hairs The leaves are cordate, oblong, obtuse, crenate and very downy on both surfaces The petioles are as large as the leaf, the stipules are linear measuring nearly half the length of the petiole The peduncles occur near the flower, the lower one is distant and is longer than the petioles, and the upper one is very short The flowers are small and white and appear during the rainy season in all species The root of the different species of sida are 2 to 5 inches long, about ¼ inch in diameter and the stock is woody and fibrous The bark is of a light yellowish brown colour If properly cultivated, the plant may grow as big as hemp or jute plant and produces a strong fibre

Uses in the indigenous medicine

The roots, leaves and seeds are all used in medicine and have a slightly bitterish taste The roots of all the species are regarded as cooling, astringent, stomachic and tonic An infusion made from them is given in nervous and urinary diseases and also in disorders of the blood and bile They are considered aromatic bitters having febrifuge, demulcent and diuretic properties The seeds are considered to be aphrodisiac and are used in gonorrhœa, cystitis and are also given for colic and tenesmus The leaves are used in ophthalmia The root juice is used for healing wounds, the juice of the whole plant is also used in rheumatism and spermatorrhœa Made into a paste with juice of palmyra tree it is applied locally in elephantiasis A decoction of the root and ginger is given in intermittent and other fevers attended with shivering fits The root bark powder is given with milk and sugar in persons suffering from frequent micturition and leucorrhœa In many nervous diseases, e g, hemiplegia, facial paralysis, headache, etc, the root is used either by itself or in combination

with asafœtida and rock salt It may be given internally and an oil called 'Balataila' prepared from a strong decoction of the drug mixed with milk and sesame oil is used as external application

Besides the above medicinal properties, the plant has got a great commercial importance on account of the fine white fibre it yields The cellulose content of this fibre is 83 per cent as against 75 per cent in jute and in the opinion of many experts no fibre of modern times affords better hopes of success than sida as a substitute for flax

Chemical examination

Sida cordifolia was analysed many years ago (1890) and was said to contain '*asparagin*' A perusal of the literature shows that no detailed or systematic study of the nature of the chemical constituents present in the plant has been carried out The drug was analysed in the Department of Chemistry by Dr Sudhamoy Ghosh and Mr Ashutosh Dutt and the following is a summary of the work, details of which will appear in a separate paper The air-dried material was secured locally and was identified by Prof E N Ghosh, M D, as *Sida cordifolia,* Linn (Bela) The whole plant (including leaves, seeds, stems and roots) was used in the present investigation

A preliminary examination showed the presence of alkaloids and a quantitative estimation showed their occurrence to the extent of 0 085 per cent of the whole plant as an average of 5 analyses The seeds were found to contain about 4 times more alkaloid than either the stems, roots or leaves

A systematic examination of the drug by extraction with different solvents showed the presence of the following (1) fatty oil, phytosterol, etc , (2) resins and resin acids, (3) alkaloids There were no glucosides or tannins The alkaloid was isolated by extracting about 120 kilograms of the coarsely powdered material repeatedly with rectified spirit The alcohol was recovered under reduced pressure and the residue repeatedly taken up with dilute hydrochloric acid The acid aqueous extract was then freed from oily matter, made alkaline with ammonia and the alkaloid extracted with chloroform The crude alkaloid thus obtained was purified by a series of long and tedious processes until the substance was obtained in a crystalline condition The hydrochloride was obtained as colourless needles freely soluble in water but sparingly soluble in absolute alcohol The yield was only about 2 grammes

Pharmacological action of the sida alkaloid

External action

The alkaloid has no irritant action on the intact skin When applied to the mucous membrane it produces well-marked vaso-constriction Injections of 1 to 2 per cent solutions in the thigh muscles of a cat do not produce any marked congestion, œdema or necrosis at the site of injection Instillation of a 1 per cent solution into the conjunctiva produces no anæsthesia of the cornea but slight dilatation of the pupil is observed

The alkaloid has a distinct action on the undifferentiated protoplasm The paramœcia are killed immediately in 1 in 100 solution, in 1 in 1,000 solution they are killed in 1½ minutes, in 1 in 2,000 solution in 5½ minutes, and in 1 in 5,000 solution in 14 minutes

Digestive system

The movements of the small intestine *in situ* are markedly inhibited by intravenous injection of the alkaloid Graph 1, *c*, shows this effect very clearly The tone of the muscle is decreased and there is generally a complete cessation of intestinal movements which lasts for 1 to 2 hours Administration of pilocarpine after the alkaloid revives the peristalsis, and pituitrin and barium chloride produce their usual effects The seat of action, therefore, is not the vagal nerve endings, nor the involuntary muscle fibres but in all probability the sympathetic nerve endings Perfusion of isolated pieces of the intestine with 1 in 50,000 of the alkaloid in Fleisch solution having a pH of 7 2 produces a marked inhibition of the movements (Graph 1, *f*) A curious fact noticed is that very small doses, such as 1/20 mg, while they may produce little or no effect on the blood-pressure temporarily increase the tone and movements of the intestine (Graph 1, *c*), possibly due to transient stimulation of vagal endings Injections of sida alkaloid after a dose of pilocarpine only partly remove the effect of pilocarpine from the gut (Graph 2, *a*) This is probably due to stimulation of the sympathetic by sida alkaloid outbalancing the pilocarpine action on the vagus

The effects on the intestinal volume are depicted in Graph 1, *a* It will be seen that there is at first a slight reduction in volume followed by a slight increase

All these experiments show that the action of the new alkaloid on the gut closely resembles a sympathomimetic base such as ephedrine The only difference noticed is that to a greater or lesser extent it removes the effects of pilocarpine on the intestine whereas according to Chen and Schmidt ephedrine has no such action The initial constricting effect of the alkaloid on the vessels is shown by a preliminary decrease of the intestinal volume, but it is soon compensated for by a passive dilatation produced by the powerful action of the heart

Circulatory system

Action on blood-pressure—Graph 1, *d*, shows the effect produced by the alkaloid on the blood-pressure taken from the carotid artery of a cat A sharp and a well-marked rise is produced lasting from 10 to 20 minutes, even half an hour in some animals In almost every experiment there is a rise of about 20 to 40 mm of mercury and in more sensitive animals as much as 70 or 80 mm of mercury This rise is produced in the decerebrated animals (Graph 1, *a*) and after the section of the vagi or administration of atropine Besides this pilocarpine produces its usual effect before or after the administration of sida alkaloid All these experiments tend to show that neither the

centre in the medulla nor the peripheral vagal mechanism are concerned in this effect

On the other hand the pressor effects produced by the drug are considerably reduced after sufficiently large doses of nicotine are given to paralyse the ganglion cells on the course of the sympathetic If the vasomotor nerve endings are paralysed by repeated injections of small doses of ergotoxine, administration of sida alkaloid produces no rise of blood-pressure The pressor action, therefore, appears to be chiefly produced by stimulation of the sympathetic A remarkable fact observed was that with very small doses such as 1/40 or 1/20 mg there was no rise of blood-pressure but in some cases actually a fall was observed (Graph 1, *b*) This phenomena has also been observed in case of adrenaline

Myocardiograph and cardiometer experiments—Injections of small doses of sida alkaloid (0 5 to 2 0 mg) produce marked acceleration of the heart beat The force and frequency of the beats are decidedly increased, both the auricles and the ventricles being affected (Graph 3, *a*) Frequently the ventricles after initial stimulation show slight dilatation which is more evident with larger doses This dilatation is no doubt due to increased peripheral resistance produced by constriction of the peripheral vessels After repeated and increasing doses of the alkaloid the force of contraction of both the auricles and ventricles appears to be decreased and the auricles show this effect earlier than the ventricles

The volume of the heart as recorded by the cardiometer shows transitory reduction followed by a slight increase

Isolated heart—Hearts of kittens and rabbits were perfused in the usual manner through the aorta with oxygenated Locke's solution having a pH of 7 2 and a temperature of 37 5°C In dilutions of 1 in 500,000 to 1 in 1,000,000, sida alkaloid produced a definite and well-marked increase in the force and frequency of the beats in very much the same way as powerful sympathomimetic bases such as adrenaline and ephedrine A perusal of Graph 2, *c* and *d*, will show that the effect produced by the sida alkaloid closely resembled that of ephedrine in similar dilutions That the action of this alkaloid was of sympathetic origin was shown by the fact that it was greatly decreased after the heart was perfused with ergotoxine, and that it remained unaltered after atropine It was further observed that when the heart was beating irregularly after repeated injections of atropine or after toxic doses of drugs which act on the myocardium, administration of this alkaloid made the heart beats regular and more powerful Perfusion of an isolated heart with 1—40,000 dilutions of the sida alkaloid increased the coronary flow by about 30 per cent Similar effects are produced by ephedrine

Action on the myocardium—It was observed that after repeated injections of large doses of sida alkaloid in an intact animal whose blood-pressure was being recorded, the systemic pressure instead of rising showed a fall of a short duration If at this stage an injection of adrenaline was given, the usual rise

of blood-pressure was produced and nicotine also produced its usual action In myocardiograph experiments also the force of contraction of both the auricles and the ventricles was decreased after repeated large doses, but injections of adrenaline produced the usual stimulation Isolated hearts of kittens after repeated perfusion with 1—20,000 or 1—10,000 solutions of sida alkaloid showed weakening but an injection of adrenaline at this stage produced the usual acceleration All these experiments support the view that in large doses at any rate, the alkaloid has a direct depressing action on the myocardium

The effects produced on the circulatory system are not appreciably altered if the animals are decerebrated, thus showing that the centres in the medulla are not involved The effects also remain unaltered if the vagal endings are previously paralysed with atropine After paralysing the sympathetic terminals, however, with repeated doses of ergotoxine, the sida alkaloid in small doses produced little or no increase in the force of the heart beat and no rise of blood-pressure whatever This shows the alkaloid in small doses has no stimulant action on the myocardium

Blood vessels—Small doses of the alkaloid were injected directly into one of the branches of the mesenteric artery, going to a selected loop of the gut whose volume was being recorded by an oncometer There was a marked decrease in the volume indicating constriction of the blood vessels (Graph 2, *e*) Further, if the mesenteric vessels were perfused with normal saline and the volume of the outgoing fluid was recorded addition of the alkaloid markedly decreased the outflow of fluid showing that the vessels were constricted If, however, the perfusion was done after sufficient doses of ergotoxine had been given to paralyse the vasomotor nerve endings, this constriction was absent These experiments show that the alkaloid acted mainly on the vasomotor nerve endings Injections of varying doses up to 2 mg of the alkaloid into the general circulation increased the limb volume (Graph 2, *h*) Also injections of 0 2 mg of the alkaloid directly into the femoral artery of the limb which had been put in a suitable oncometer, showed a slight increase in the volume (Graph 2, *f*) It would appear from these experiments that the dilatation of the vessels was partially active

The volume of the kidney and the spleen shows a rise corresponding to the rise of blood-pressure (Graph 1, *b*, *e*)

Respiratory system

The effect on the respiratory system was studied by observing the intratracheal and intrapleural pressures, and both these showed a well-marked dilatation of the bronchioles It will be seen that the intratracheal pressure falls and the intrapleural pressure rises after the alkaloid (Graph 3, *b*) After administration of pilocarpine the action of the alkaloid on the bronchial muscles is intensified in much the same way as ephedrine

The effect of the alkaloid on the nasal mucous membrane was observed in chloralosed cats, the technique described by King and Pak being employed

An intravenous injection of sida alkaloid as well as its local application of its solution produced a marked constriction of the nasal mucous membrane (Graph 1, *d*) such as is produced by the sympathomimetic bases such as adrenaline and ephedrine

Genito-urinary system

The alkaloid produced a definite increase in the kidney volume (Graph 1, *e*), accompanied by an increase in the urine secretion

The non-pregnant uterus *in situ* in the cat was relaxed by intravenous injections of the alkaloid (Graph 2, *b*) The isolated non-pregnant and virgin uterii of rabbits suspended in Fleisch's solution at a pH of 7 2 showed a well-marked increase in the tone in dilutions of 1 in 50,000 of the alkaloid (Graph 2, *g*) *In order to see if* the effect was due to stimulation of the sympathetic, sufficient doses of ergotoxine were added to the uterine bath There was no contraction

Discussion

A study of the experimental data given above is sufficient to show that the most important effects produced by sida alkaloid are a well-marked and persistent rise of blood-pressure, a marked inhibition of the tone and movements of the intestines, and a relaxation of the bronchial musculature These are the common effects produced by all sympathomimetic bases such as adrenaline, ephedrine, etc A careful perusal of the effects produced on other tissues further bears out this action It is also clear that the action is not so powerful as adrenaline but bears close resemblance *to that* of ephedrine, the alkaloid obtained from *E vulgaris* Further, we have seen that the melting point, specific rotation, the platinic and gold chloride tests, the molecular weight determinations and the biuret reaction were approximately the same as those of ephedrine The filtrate from the crystalline hydrochloride contained some more alkaloid which did not crystallize out but which from its pharmacological reactions appeared to be a mixture of ephedrine and one or more of its isomers Unfortunately owing to the alkaloid content of *S cordifolia* being very small sufficient quantities could not be isolated to enable the chemists to work out fully its chemical constitution This is now being done by the Department of Chemistry but in the mean time a comparative study of the effects of this alkaloid and other sympathomimetic bases, such as ephedrine, pseudo-ephedrine and adrenaline on the animals tissue, shows that with the exception of a few minor details, which may possibly be attributed to impurities, the action of sida alkaloid approximates more closely to ephedrine than other bases

Sida alkaloid acts mainly on the sympathetic nerve endings but it appears to have some effect on the sympathetic ganglia The musculature of the heart and the blood vessels is slightly affected if at all This alkaloid and ephedrine produce almost identical effects on the circulatory, gastro-intestinal, respiratory and genito-urinary systems In case of the heart the only variation is that

TABLE

Organs acted on	*Sida alkaloid*	*Ephedrine*	*Pseudo-ephedrine*	*Adrenaline*
Intestinal movement	Persistent inhibition	Persistent inhibition	Inhibition but movements soon reappear	Inhibition
Intestinal volume	Slight constriction followed by slight dilatation	Slight constriction followed by slight dilatation	Unaffected or slight constriction or slight dilatation	Constriction followed by dilatation
Blood-pressure	Sharp and persistent rise	Sharp and persistent rise	Smaller rise	Sharp rise quickly followed by a fall sometimes to below normal
Pulmonary pressure	Slight increase	Slight increase	Slight increase	Marked increase
Myocardium	Depressed after repeated toxic doses	Depressed after repeated toxic doses	Stimulated	Little or no action
Coronary outflow	Increased with large doses	Increased with large doses		Increased
After ergotoxine	No rise of blood-pressure Little or no effects on the auricles or the ventricles	No rise of blood-pressure Both auricles and ventricles show slight depression	Slight rise of blood-pressure Both auricles and ventricles show stimulation	Blood-pressure falls
Mesenteric vessels	Constriction followed by dilatation	Constriction followed by dilatation	Constriction or slight dilatation	Constriction followed by dilatation
Limb vessels	Slight dilatation	Slight dilatation	Slight dilatation	Slight dilatation
Respiration	Relaxation of bronchioles	Relaxation of bronchioles	Relaxation of bronchioles	Relaxation of bronchioles
Uterus ..	Rabbit—contraction	Rabbit—contraction	Rabbit—little or no change	Rabbit—contraction
	Cat—relaxation of non-pregnant	Cat—relaxation of non-pregnant	Cat—relaxation of non-pregnant	Cat—relaxation of non-pregnant
Seat of action	Chiefly sympathetic endings and ganglia	Mainly on sympathetic endings and ganglia	Acts on sympathetic endings, ganglia and muscle	Acts chiefly on the sympathetic nerve endings

after ergotoxine, this alkaloid instead of producing slight depression of the myocardium as is the case with ephedrine, produces either no action whatever or a slight tendency to stimulation, which is seen in case of pseudo-ephedrine Ephedrine also has no action on the plain muscles of the blood vessels while sida alkaloid has been shown to have a weak action A similarity in the action of sida alkaloid and ephedrine is also brought out in their action on the splanchnic and peripheral vessels On the gastro-intestinal tract though sida alkaloid has an ephedrine-like action, it was noticed that it slowly removes the effect of pilocarpine on the gut, while it has been shown by Chen and Schmidt that ephedrine has no such action

The most interesting finding of this investigation is the presence of sympathomimetic alkaloids in plants of widely divergent nature *Ephedra vulgaris* from which ephedrine is obtained belongs to the gymnosperms and *Sida cordifolia* belongs to the angiosperms, two entirely different divisions of the vegetable kingdom

Summary and conclusion

1 The active principle of *Sida cordifolia* (*Bala*) is an alkaloid with well-marked sympathomimetic action A detailed study of the pharmacological action of this alkaloid on various tissues and organs shows that its action closely resembles ephedrine

2 Further work on the chemistry of this alkaloid is in hand with a view to determine if the alkaloid is ephedrine or a closely allied compound

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Explanation of Plate XXX

(Graph 1)

a Male cat, 2,880 gs., decerebrated. Intestinal volume and carotid blood-pressure, the vertical line represents centimetre scale. 2 mg. of sida alkaloid produces rapid and persistent rise of blood-pressure and increase of intestinal volume.

b Male cat, 2,560 gs., chloralose. 1/20 mg. of sida alkaloid into the femoral vein produces fall of blood-pressure and increase of spleen volume.

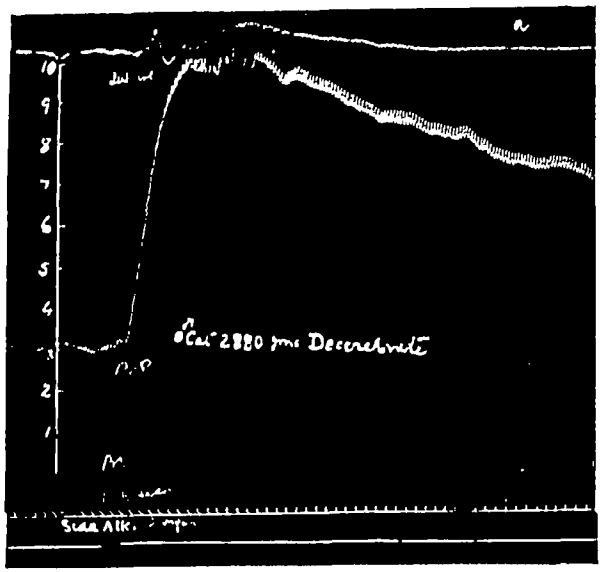
c Female cat, 2,100 gs., chloralose. Intestinal movement *in situ*. Upstroke contraction and downstroke relaxation. 1/20 mg. of sida alkaloid produces increase of muscular tone and intestinal movements.

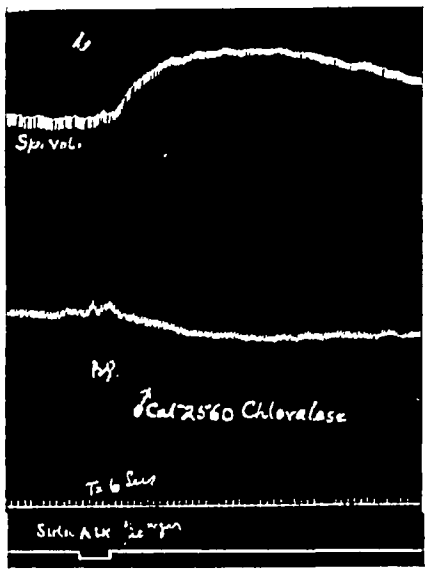
d Female cat, 2,100 gs., chloralose. 0·5 mg. of sida alkaloid intravenously produces marked contraction of the nasal mucous membrane.

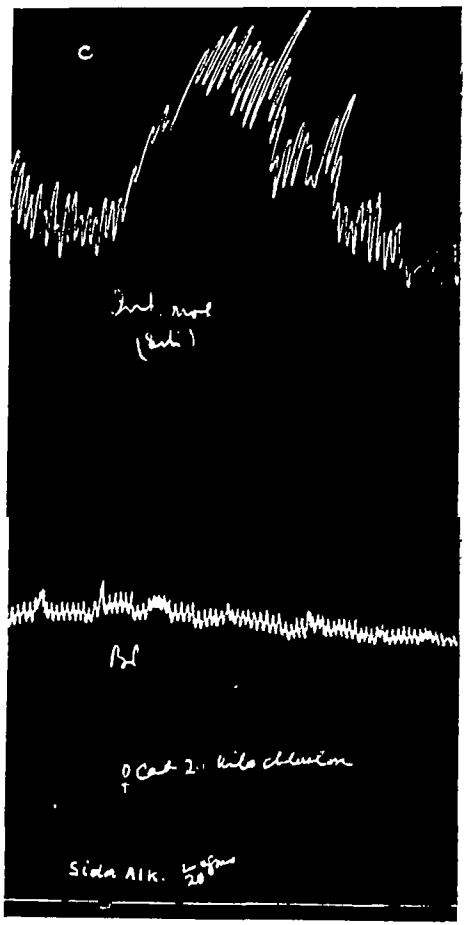
e Female cat, 2,380 gs., decerebrated. 0·25 mg. of sida alkaloid produces increase of kidney volume and blood-pressure and inhibits intestinal movements *in situ*.

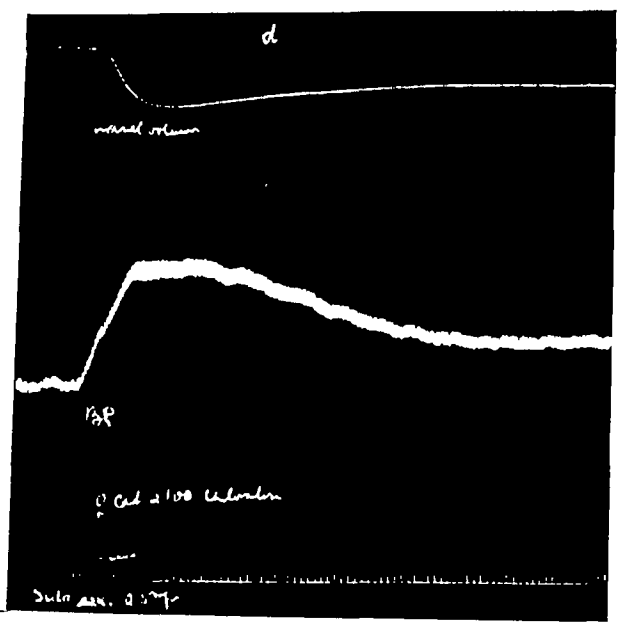
f Perfusion of isolated cat's intestine. Note the decrease of intestinal movements and tone by 1 in 50,000 of sida alkaloid.

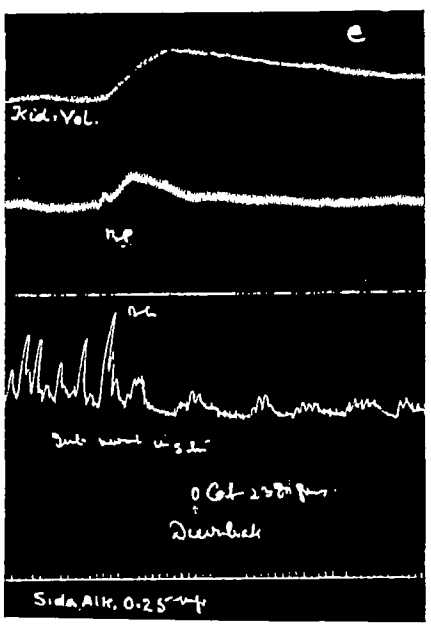
PLATE XXX
(Graph 1)

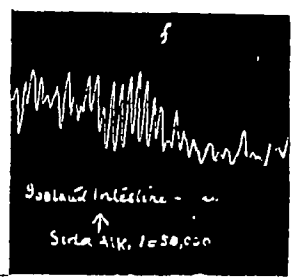












Explanation of Plate XXXI

(Graph 2)

a Male cat, 2,140 gs., chloralose. Intestinal movements *in situ* and blood-pressure. 0·5 mg. of pilocarpine was first injected and later 1·0 mg. of sida alkaloid. The effect of pilocarpine on the gut is partly removed.

b Female cat, non-pregnant, chloralose. 1 mg. of sida alkaloid produces temporary inhibition of uterine contractions *in situ*.

c—d Perfusion of isolated heart of kitten. 1 in 500,000 of sida alkaloid and ephedrine produce a marked stimulation of the frequency, force and amplitude of the beats. Upstroke diastole and downstroke systole.

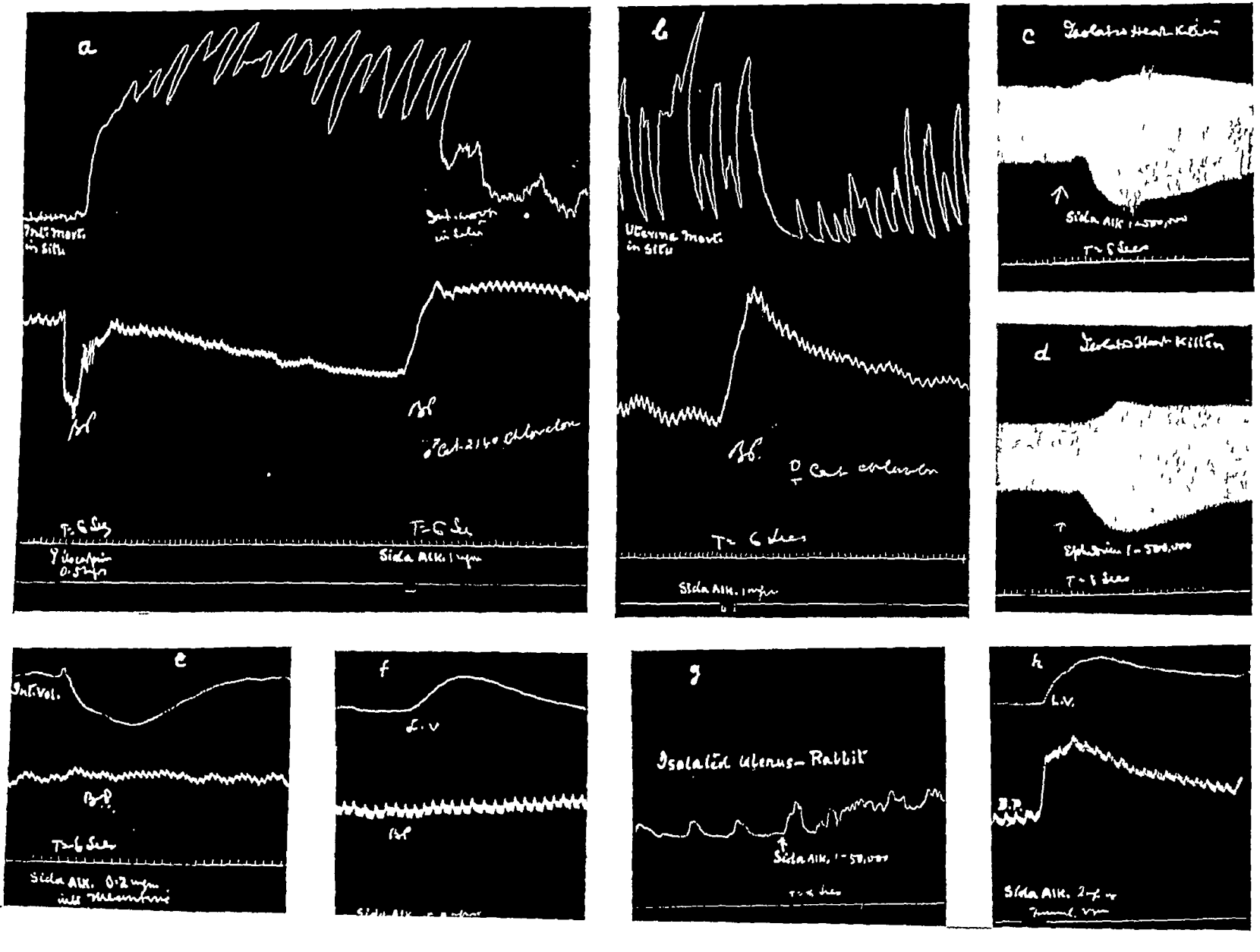
e Male cat, 2,160 gs., chloralose. Injection of 0·2 mg. of sida alkaloid into the mesenteric artery produces a marked decrease in the intestinal volume (upper tracing).

f Male cat, 2,110 gs., chloralose. Injection of 0·2 mg. of sida alkaloid into the femoral artery produces a marked increase of the limb volume (upper tracing).

g Isolated rabbit's uterus. 1 in 50,000 of sida alkaloid produces a marked increase of tone.

h Male cat, 2,080 gs., chloralose. 2 mg. of sida alkaloid into the femoral vein produces increase in the limb volume and the blood-pressure.

PLATE XXXI
(Graph 2)



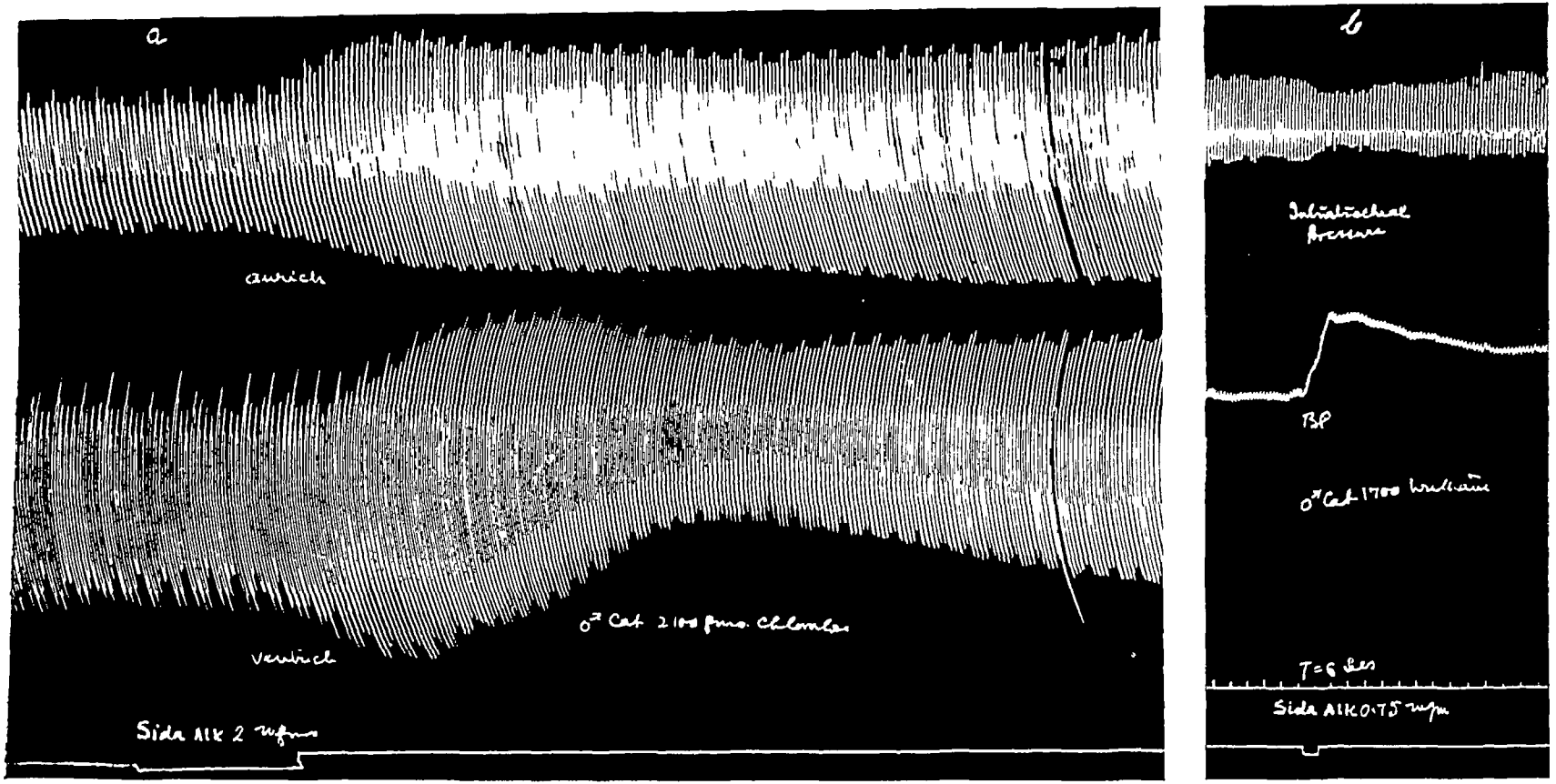
Explanation of Plate XXXII

(Graph 3)

a Male cat, 2,100 gs, chloralose The upper tracing auricular and the lower ventricular movements Upstroke diastole and downstroke systole 2 mg of sida alkaloid into the femoral vein produce increase of force and frequency of the beat The rate was increased by 35 per cent

b Male cat, 1,700 gs, chloralose 0 75 mg of sida alkaloid produces dilatation of the bronchioles

PLATE XXXII
(Graph 3)



A FEW ATYPICAL CASES OF MYCETOMA

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THE following cases of mycetoma are described because we think that they are peculiar in type and in situation

(1) *Specimen No* 1-*P* *Section No* 3083 (Plate XXXIII, fig 1, and Plate XXXIV, figs 1 and 2)

It is a mass removed from the sacrum of a Hindu male aged 20 years and of about 10 years' duration The only available information regarding the case is from the surgeon's notes, which are as follows 'A partly cystic and partly solid growth situated over the lower end of the sacrum with extensive prolongations distally into the glutæal region The growth was very adherent to the skin but was easily mobilized with the deep fascia from the deeper structures There was one large cyst about the size of a large lemon and many smaller ones of varying sizes Some of these ruptured on detaching and a dirty grumous fluid containing a large number of hard black granules escaped As far as could be seen the growth was completely removed'

Naked eye appearances —

The growth consists of an elongated mass of round cysts held together by connective tissue The cyst at the top of the specimen is somewhat smaller than a lime and the others, six in number, are of the size of a marble each On section, the cyst wall consists, externally of a dark tissue (the connective tissue holding them together) and internally of a yellowish brown material which lines the interior to a varying depth—that in the lowest cyst being very

abundant almost filling the cavity Embedded in this ochroid material are seen dark gunpowder-like granules—the fungal masses

Microscopical appearances —

The section shows a dense fibrous wall lined by a mass of granulation tissue consisting of young capillaries, polymorphonuclear leucocytes, small round cells, plasma cells fibroblasts, and endothelial cells containing dark brown pigment Internal to this zone, are seen dark brown masses of fungi embedded in a mass of polymorphonuclear leucocytes The fungal mass appears structureless and the hyphæ are not distinct

To the naked eye the granules appear as dark gritty gunpowder-like masses *On treating these with caustic potash, the mycelia may be easily* separated and studied The fungus is seen to be composed of segments 9 5μ to 10μ long and 3μ to 3 5μ broad The wall is distinct and inside the protoplasm are seen intercalary spores The hyphæ show dichotomous branching Here and there, specially towards the ends of the hyphæ, the segments are broad and expanded into spade-like bulgings

(2) *Specimen No 3-P Section No* 3041 (Plate XXXIII, fig 2, and Plate XXXIV, figs 3 to 6)

This is a mass removed from the sole of the foot Unfortunately there is no history available except that 'It is a tumour of the sole of the foot with dark granules Incised by a barber and septic glands groin are present'

Naked eye appearances —

The specimen consists of three spherical masses The one on the left is slightly larger than a marble with a distinct capsule containing a whitish material in the middle of which are embedded dark gritty granules The other mass is similar to the former in size and appearance The third is a similar mass but smaller in size

Microscopical appearances —

The section shows a dense fibrous capsule containing darkly staining masses of fungi of varying sizes embedded in a mass of chronic inflammatory granulation tissue These masses are dark brown in colour and structureless These are surrounded by polymorphonuclear leucocytes and fibrous tissue The tissue intervening between these masses is composed of connective tissue fibres, plasma cells, small round cells and endothelial cells, some of them containing dark brown pigment Large multinucleated giant cells are also seen in numbers

The fungus consists of branching segmented hyphæ with well-defined walls containing granules The segments are about 12μ to 13μ long and 3μ to 3 5μ broad Some of these segments, specially the terminal ones, are irregularly broad and expanded

(3) *Specimen No* 4-*P Section No* 3029 (Plate XXXIII, fig 3, and Plate XXXV, figs 1 to 4)

The specimen is a mass removed from the dorsum of the foot Unfortunately very little clinical history is available except that the patient was an adult male aged about 35 years and a native of Ramnad district It was of

4 years' duration and was excised for as a fibroma and sent with the following description 'a lobulated tumour hard to feel and containing brownish fluid with dark granules in it and recurred after operation'

Naked eye appearances —

The section shows a dense fibrous capsule lined by chronic inflammatory granulation tissue composed of capillaries, fibroblasts, plasma cells, and endothelial cells Inside are seen dark brown fungal masses whose margins are darker than the centre In the central portions are seen segmented and branched hyphæ These masses are surrounded by polymorphonuclear leucocytes and a clear homogeneous exudation

The fungus consists of branching segmented hyphæ with well-defined walls and intercalary spores The segments are 12 5 μ to 13 μ long and 3 μ to 3 5 μ broad Broad spade-like expansion of some of the segments are seen in large numbers, especially the terminal ones

(4) *Specimen No A Section No* 8025 (Plate XXXVI, figs 1 to 4)

The specimen is an amputated foot from a gardener aged about 40 years and the condition was of four years' duration

Naked eye appearances —

The specimen consists of the foot and the ankle joint The joint is swollen and all round it are seen numerous sinuses discharging pus and white granules There are also a few sinuses on the foot The granules are small, somewhat larger than a pin-head, soft and pale cream in colour and the discharge has a heavy fishy smell On section small areas of suppuration of the size of a pin-head are seen all over, specially in the periarticular ligaments and the connective tissues The ligaments are degenerate and disorganized The tarsal bones are carious and show large areas of suppuration

Microscopical appearances —

The section shows chronic inflammatory granulation tissue with areas of suppuration in which are seen masses of fungi These fungal masses consist of thin hyphæ densely packed in the centre with "club-shaped processes at the periphery, having the 'ray-fungus' appearance" The clubs are Gram-negative while the hyphæ are Gram-positive Both the clubs and the hyphæ are moderately acid-fast, being decolourised by 20 per cent sulphuric acid but resists 5 per cent acid

The fungus consists of thin branching hair-like hyphæ 1μ to 1 5 μ thick, there being no differentiation between the wall and the protoplasm

Attempts at culturing the organism both aerobically and anaerobically were unsuccessful

(5) *Specimen No B Section No* 9061 (Plate XXXIII, fig 4)

The specimen was taken from the dorsum of the foot of a Hindu female It was a circumscribed tumour of the size of a lime

Naked eye appearances —

The specimen is an oval mass of the size of a lime At one part the skin of the foot is adherent to it On section it is of a pale bluish white colour and numerous yellowish brown circular areas of the size of a split pea are seen scattered all over it Here and there are reddish vascular areas It has a distinct capsule and is firm in consistency

Microscopical appearances —

The section shows chronic inflammatory granulation tissue with areas of suppuration in which are masses of fungi The filaments of the fungi are densely packed in the centre but are clearly seen at the periphery These are very thin and hair-like, non-segmented but branched They are Gram-positive but not acid-fast

(6) *Specimen No C Section No* 9332

Naked eye appearances —

The specimen consists of the foot and ankle All round the ankle joint are numerous sinuses in which are seen tiny white granules The foot is free of sinuses

Microscopical appearances —

The section consists of chronic inflammatory granulation tissue with areas of suppuration in which are seen masses of fungi These consist of a central network of hair-like filaments and club-shaped processes radiating from the periphery, typical of the 'ray-fungus' Around these are polymorphonuclear leucocytes, and young blood vessels Beyond is fibrous tissue infiltrated with plasma cells and round cells

(7) *Specimen No* 2-*P Section No* 3079

The specimen was from the knee-joint of a young man of 19 years It was a tumour of the size of an orange below and to the outer side of the knee containing brown thick fluid and black sandy granules The base was adherent to the capsule and the synovial membrane The specimen consists of a portion of the synovial membrane in which are embedded numerous dark inky granules

Microscopical appearances —

The section shows a dense fibrous lamina with chronic inflammatory granulation tissue in which are seen numerous brown masses of fungi All around are seen numerous round cells, plasma cells, and large multi nucleated giant cells The fungus is of the maduromycotic variety and has the same appearances as the previous ones The terminal spade-like expansions are also clearly seen

Discussion

The points of special interest in these cases are the peculiar situations and the appearances of these lesions The most common site for mycetoma is the sole of the foot and occasionally the dorsum But situations like the knee

joint and the gluteal region are certainly unusual though in the latter place other fungi than the Maduromyces have been described Again, usually one associates with the term 'mycetoma' chronic inflammatory lesions riddled with sinuses in which are seen fungal masses But localized spherical capsulated tumours, such as some of the above, are we think very unusual One should not be surprised at the appearance of the lesions, for the fibrous tissue response on the part of the tissue to the chronic inflammation results in a dense capsule—an attempt at limiting the spread of the disease Another point of interest is the presence of the spade-like expansions of some of the hyphæl segments Are these evidences of degeneration of the fungi incarcerated in a hostile soil trying to limit its spread and if possible to destroy it? We have seen such expansions only in human lesions but not in cultures Again two of the above cases are caused by the 'ray-fungus,' probably *N bovis* Human infections by this fungus are not met with commonly in South India, but one should be on the look out for them

We are indebted to the Radiologist, Government General Hospital, Madras, for photographs of the specimens

Explanation of Plate XXXIII

Fig. 1 Specimen No. 1-P
" 2 Specimen No. 3-P
" 3 Specimen No. 4-P
" 4 Specimen No. B

PLATE XXXIII

Fig 1—Specimen No 1-P (*Natural size*)

Fig 2—Specimen No 3-P (*Natural size*)

Fig 4—Specimen No B (*Natural size*)

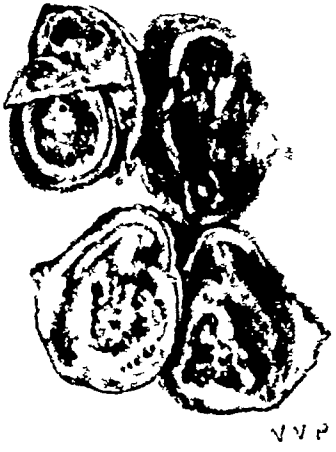

Fig 3—Specimen No 4-P (*Natural size*)

Fig 1

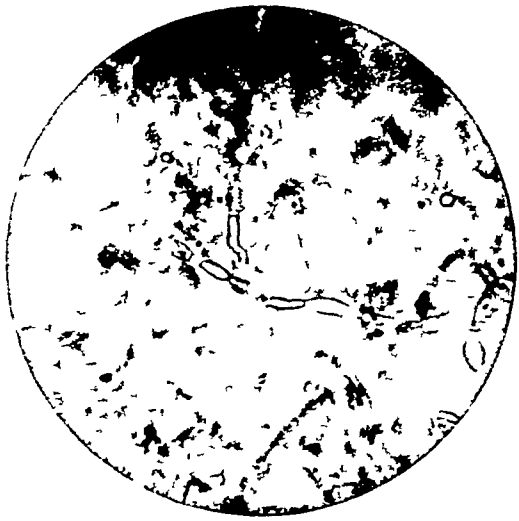

Fig 2

Fig 3

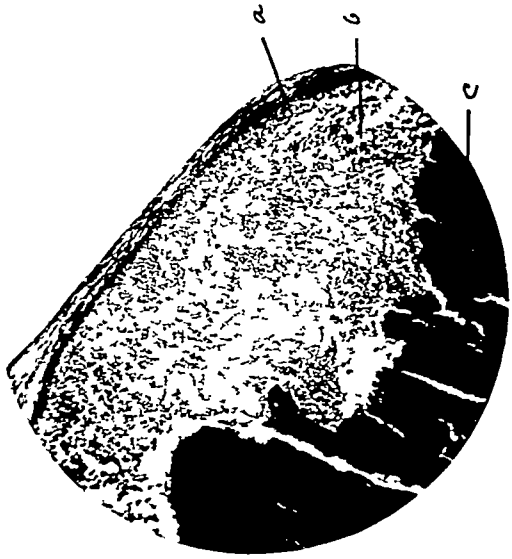

Fig 4

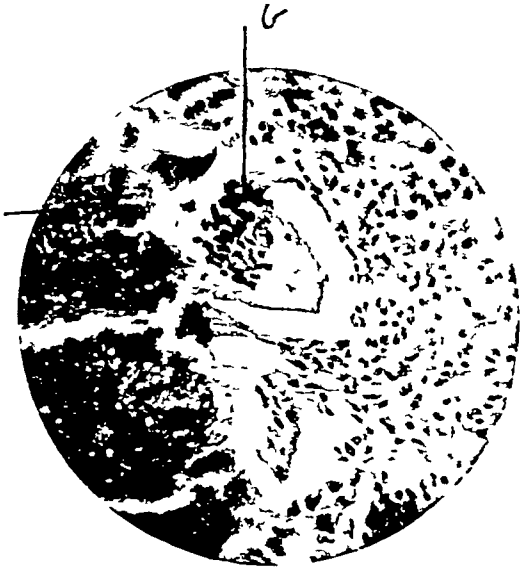

Fig 5

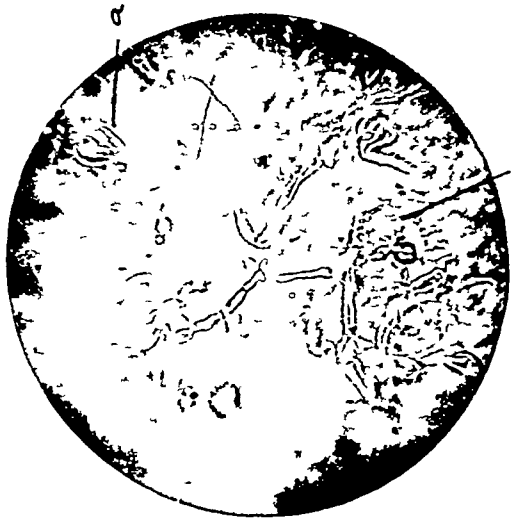

Fig 6

EXPLANATION OF PLATE XXXV

Fig 1 Photo of specimen No 4-P

„ 2 Microphoto of section (No 3029) from specimen No 4-P
(*a*) Fibrous wall (*b*) Granulation tissue (*c*) Fungus

„ 3 Higher magnification of fungal mass in same section, showing the filaments

„ 4 Microphoto of another part of same section

PLATE XXXV

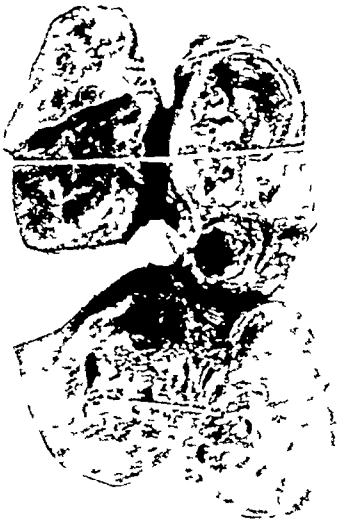

Fig 1

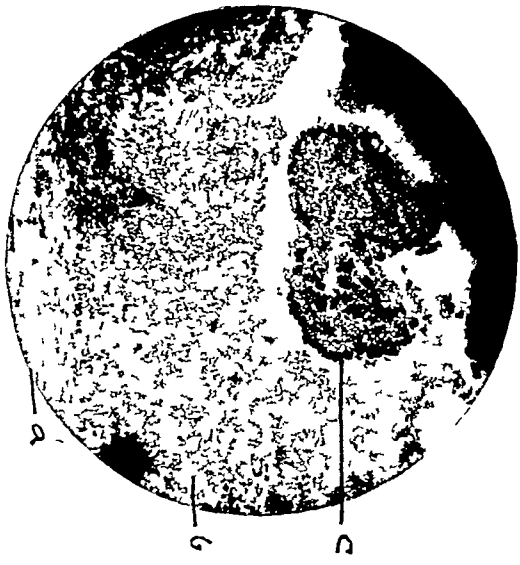

Fig 2

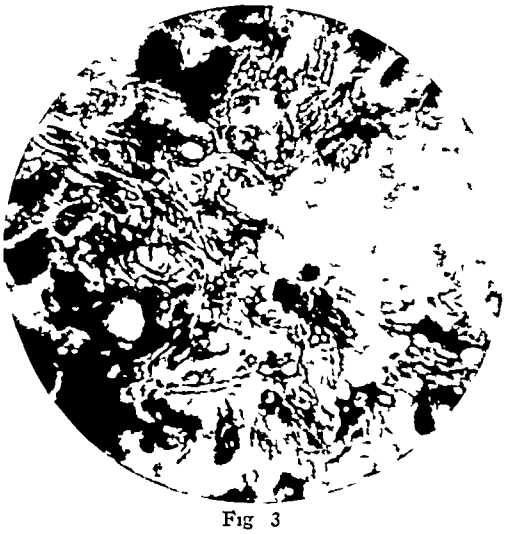

Fig 3

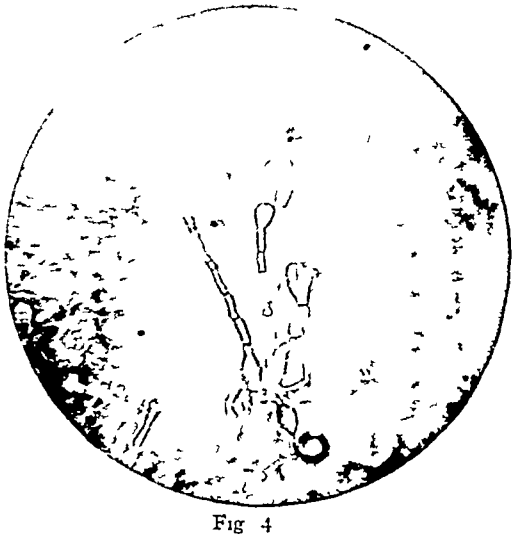

Fig 4

EXPLANATION OF PLATE XXXVI

Fig 1 Photo of specimen No A

„ 2 Photo showing cut surface of specimen No A

Figs 3 and 4 Microphotos of sections (No 8025) from specimen No A

PLATE XXXVI

Fig 1

Fig 2

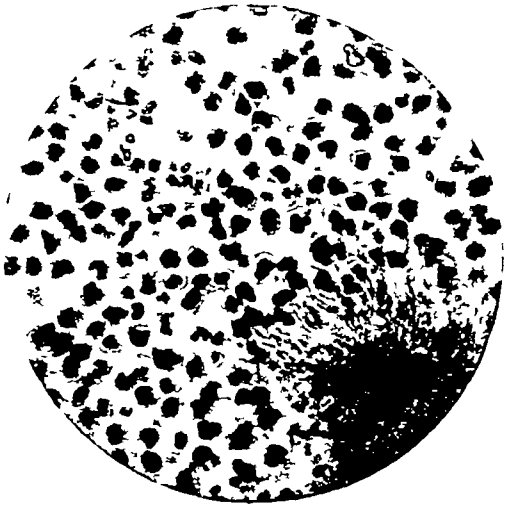

Fig 3

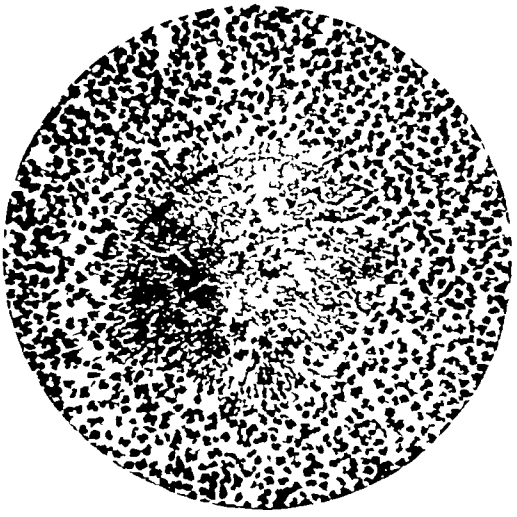

Fig 4

HEART DISEASE IN THE PUNJAB WITH SPECIAL REFERENCE TO MITRAL STENOSIS

BY

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AFFECTIONS of the heart are met with much less often in tropical and subtropical countries than in temperate regions, the difference being largely accounted for by the rarity of rheumatic fever in the former. This fever probably varies in incidence and type in different parts of the tropics. In textbooks of Tropical Medicine it receives little or no attention. Stitt (1929) states that it caused 71 deaths in Calcutta in 1911 and that in the same year there were 614 cases reported in the Gold Coast. He also points out that in the latter colony the admissions for valvular disease of the heart were not as many as the number of cases of rheumatic fever would lead to expect. Syphilis, which is often inadequately treated, plays an important part in the causation of cardiac and vascular disease in warm countries and infections endemic in tropical countries are occasionally responsible for heart lesions.

The observations recorded in this paper are published for the purpose of drawing attention to the occurrence of acquired heart disease among the inhabitants of the Punjab and to the value of certain modern methods in its diagnosis and prognosis. Thirty-five cases are dealt with, 31 of whom were treated in our wards in the Mayo Hospital, Lahore, during the period from 1st October, 1929, to the 30th April, 1930. These made up 5·6 per cent. of the total admissions to our wards during the same period. It is probable that cardiac disease is more common in the province than this figure indicates, as the poorer classes, to which the majority of our patients belonged, do not usually seek treatment for chronic conditions unless their activities are seriously

hampered Chronic heart disease therefore, often escapes attention until compensation has completely, or almost completely, broken down Ten individuals with mitral disease were admitted for conditions other than lesions of the heart, the latter being found in the course of the routine examination All patients were residents of the Punjab and all but one were Indians The exception was an Anglo-Indian who had lived the greater part of his life in the province

The cases are divided into two groups, those with definite signs of valvular disease and those in whom no such signs could be detected Their distribution according to ages and to the conditions diagnosed is given in a table Methods of examination included X-rays and electrocardiography Only one case came to autopsy (No 2, Group I) Our hospital patients are usually removed to their homes by their relatives when death is imminent and in any case post-mortem examinations are not often permitted Another defect in the analysis is the absence of bacteriological data, the obtaining of which was not feasible

Group I (No detectable valvular disease)

There are 11 cases in this group, 9 males and 2 females All had symptoms of myocardial insufficiency, viz, pain, dyspnœa, cyanosis, palpitation, or

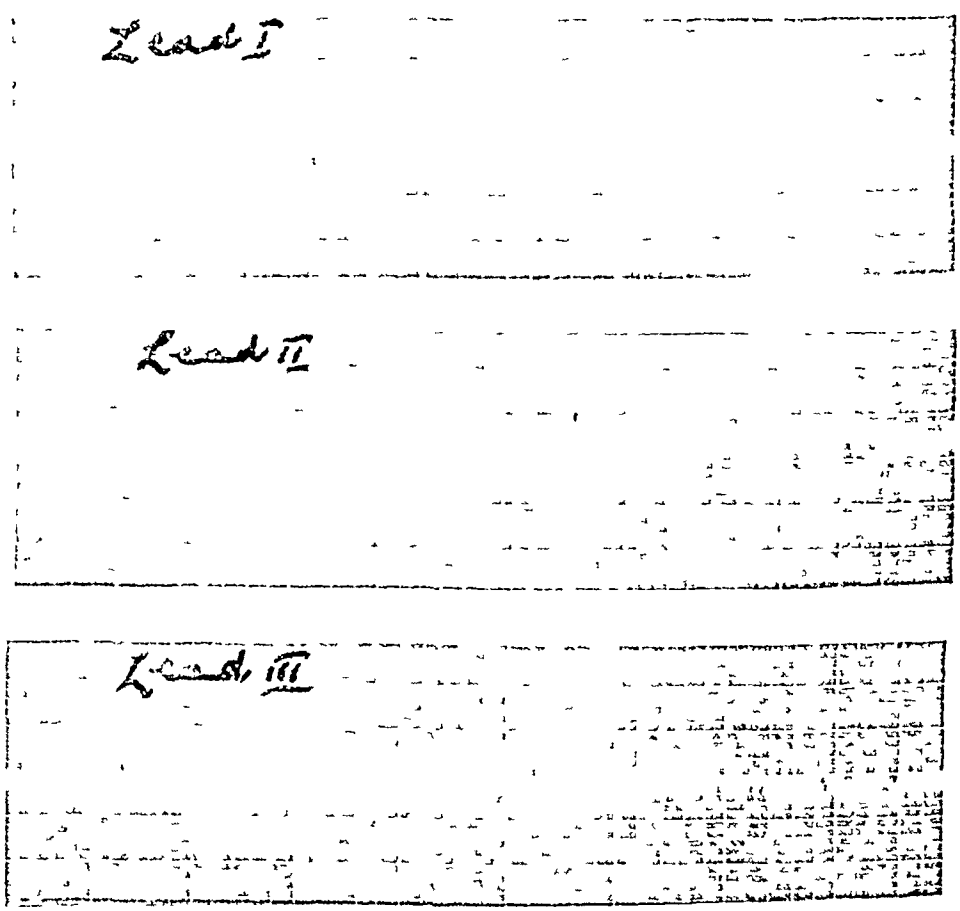


Fig 1—Patient No 5, Group I Auricular fibrillation Low voltage

chronic passive congestion, and in some there was relative incompetence of the mitral valve No 8 came under observation a week after having had a severe attack of substernal pain which radiated down both arms and was accompanied by vomiting and collapse No 3 sought relief from pain of a severe nature which was very persistent and radiated round the left side of the chest to the back The pain was undiminished by nitrites, morphia alone having any effect In both these patients the symptoms resembled those of cardiac infarction and there were marked electrocardiographic abnormalities (*vide* infra) No 5, whose auricles were fibrillating, dated his disability from an attack of plague eleven years ago In two patients the Wassermann reaction was strongly positive and one of these had a fairly large aneurysm of the descending aorta It is likely that syphilis was a causative factor in some of the others also Thus No 2 whose W R was negative gave a clear history of having had this disease Cowan and Faulds (1929) state that the W R is positive in only 75 per cent of patients in the tertiary stage, the period at which most syphilitic heart cases are seen

The information obtained from the electrocardiogram in Group I shows the importance of this method of examination in patients with symptoms

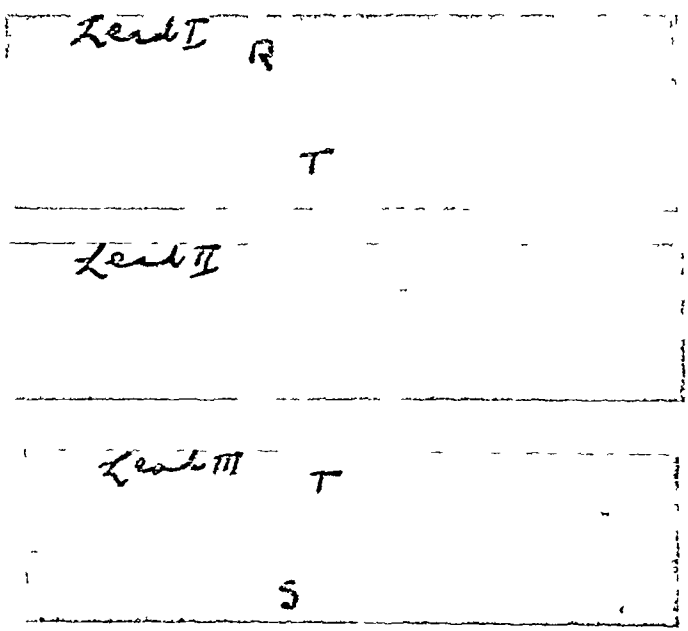


Fig 2—Patient No 3, Group I Right bundle branch block

suggestive of defective myocardium In 7 cases including the patients with auricular fibrillation (Fig 1) it gave definite evidence of myocardial disease In No 3 (Fig 2) the curves indicated a block of the right branch of the

auriculo-ventricular bundle and in Nos 2 and 10 (Figs 3 and 4) an 'intra-ventricular' block In No 2 a large aneurysm of the apical part of the left ventricle was found post-mortem In No 6 (Fig 5) there was notching and broadening of the R wave in lead III and inversion of the T wave in the same

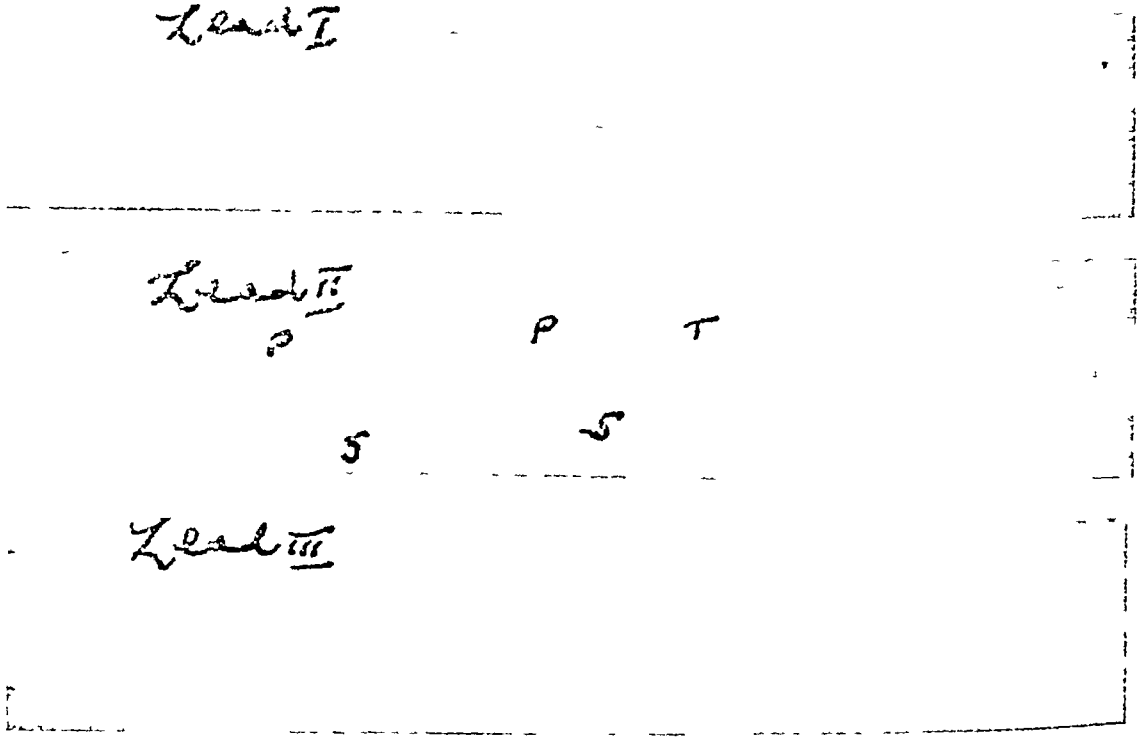


Fig 3—Patient No 2, Group I 'Intraventricular block'

lead In No 8 (Fig 6) the T wave was curved and dipped in leads II and III and the Q wave was prominent in lead III The former is a common feature in cases of coronary thrombosis and the latter was found by Levine (1929) to be of frequent occurrence in the same condition The Q wave was prominent in leads II and III in patient No 7 and in lead III in No 9 (Fig 7) We found a prominent Q wave in lead III in two other subjects, not included in the present series, in whom there were symptoms of myocardial insufficiency In general the response to treatment in this group was poor, particularly in the case of patients with electrocardiographic signs of severe myocardial involvement

Group II (Valvular disease)

Twenty-four patients, 18 males and 6 females, presented evidence of valvular lesions and in all but one (No 1) the mitral valve was affected

This exception was a case of uncomplicated aortic regurgitation In four individuals aortic regurgitation was associated with mitral disease in three with stenosis and in one with incompetence In the remaining 19 the mitral valve alone was involved 3 had signs of mitral regurgitation, 3 (including the case of

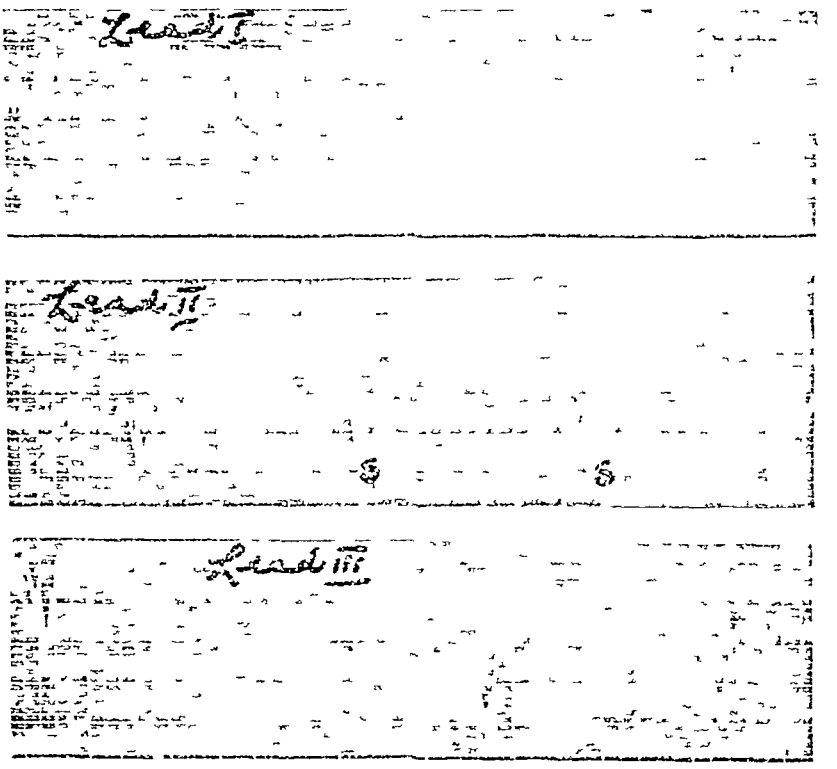


Fig 4—Patient No 10, Group I 'Intraventricular block'

auricular fibrillation) had signs of 'double mitral' disease and 13 had signs of mitral stenosis In 19 patients, therefore, there was evidence of narrowing of the mitral orifice A well-marked apical diastolic murmur, sometimes accompanied by a thrill, was heard in eleven of these In 4, including the case of auricular fibrillation, this murmur did not extend into the last part of diastole In 7 it occupied either the whole of diastole or existed only during presystole In either event the presystolic bruit was crescendo in character and terminated abruptly at the beginning of systole Occasionally it was intermittent In 3 cases a faint rumble could be heard in late diastole after exertion only and with the patient in the left lateral position A blowing systolic murmur localized at the apex or just inside it accompanied the diastolic bruit in six patients In 3 of these, Nos 11, 14 and 15, the diastolic murmur developed sometime after the systolic In five individuals however a localized apical systolic murmur alone could be detected In these a diagnosis of mitral stenosis was made

partly from the character and localization of the murmur and the quality of the first sound and partly from the X-ray appearances of the heart Cookson (1929) has drawn attention to the frequent absence of diastolic murmurs in mitral stenosis and to the value of X-rays in the diagnosis of this condition The features of diagnostic importance are enlargement of the left auricle and prominence of the pulmonary artery Skiagrams were taken in the right anterior oblique and antero-posterior positions In the former enlargement of

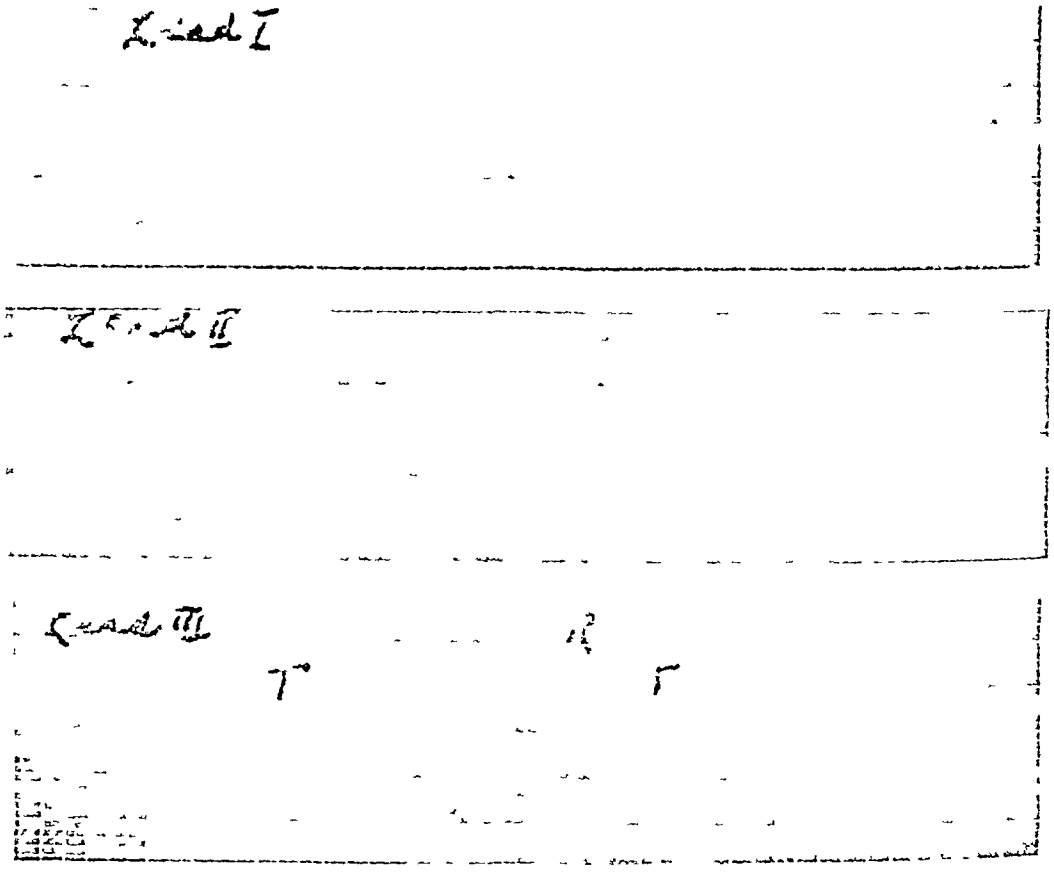


Fig 5—Patient No 6, Group I T wave hardly noticeable in leads I and II and inverted in lead III R wave notched and QRS complex broadened in lead III

the auricles is revealed by their encroachment on the retrocardiac space between the heart and the vertebral column, the left auricle being above and the right below (Plate XXXVIII, figs 1 and 2) In the antero-posterior view marked enlargement of the left auricle causes a bulging on the left side of the cardiac shadow at the upper end of the left ventricular border (Plate XXXIX, fig 3) A prominent pulmonary artery gives rise to an accentuation of the curve above this point When the latter feature is associated with enlargement of the left ventricle the left border of the heart shadow becomes a straight line (Plate XXXIX, fig 4) Cookson points out that a rounded mass of glands in the posterior mediastinum may resemble enlarged left auricle in the right anterior

oblique position and that a visible descending aorta and an enlarged root shadow may be mistaken for a prominent pulmonary artery in the antero-posterior position It is possible, however, to eliminate these conditions by noting the

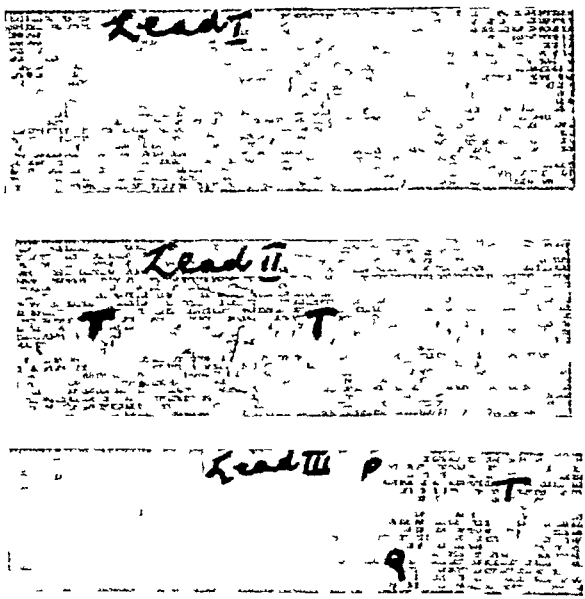


Fig 6—Patient No 8 Group I T wave curved and dipped in leads II and III QRS complex wide and low in lead II Prominent Q wave in lead III

shape, position and outline of the shadows and by taking the other signs and symptoms into account We are of opinion that this method of examination is of great assistance in the diagnosis of mitral stenosis with equivocal signs

The Wassermann reaction was strongly positive in the patient with pure aortic regurgitation and in the case with both aortic and mitral incompetence It was negative in all the others Five cases of mitral disease came under treatment with subacute multiple arthritis and 13 gave a history of having suffered from painful swollen joints Syphilis, gonorrhœa and other specific causes of joint disease were excluded as far as possible From observation of patients in the series dealt with here, as well as of others, we have come to the conclusion that in the Punjab lesions of the heart, especially of the mitral valve, often bear an ætiological relationship to a disease which in certain respects resembles the rheumatic fever of temperate climates We have seen this disease in persons without any signs of heart involvement as well as in patients with such signs It shows a strong tendency to relapse One individual (No 14), who was in hospital on two occasions, was admitted first with multiple arthritis and no indication of heart disease and subsequently, two months later, with a second

attack of arthritis and definite signs of a mitral lesion Some patients gave a history of having suffered from rheumatic attacks for years It will be noticed that the average age of patients with mitral stenosis was relatively low and for the reason already given it is probable that in some of them the valve had been affected a considerable time before they came under notice Unhealthy tonsils were found in two cases suffering from joint disease alone but tonsillectomy had no influence on the course of the illness The arthritis varies in severity Sometimes the attack starts abruptly and the temperature rises to 102° or 103°F where it remains for two or three days (Plate XXXVII, fig 1) The joints are painful and tender and synovial effusions appear When the

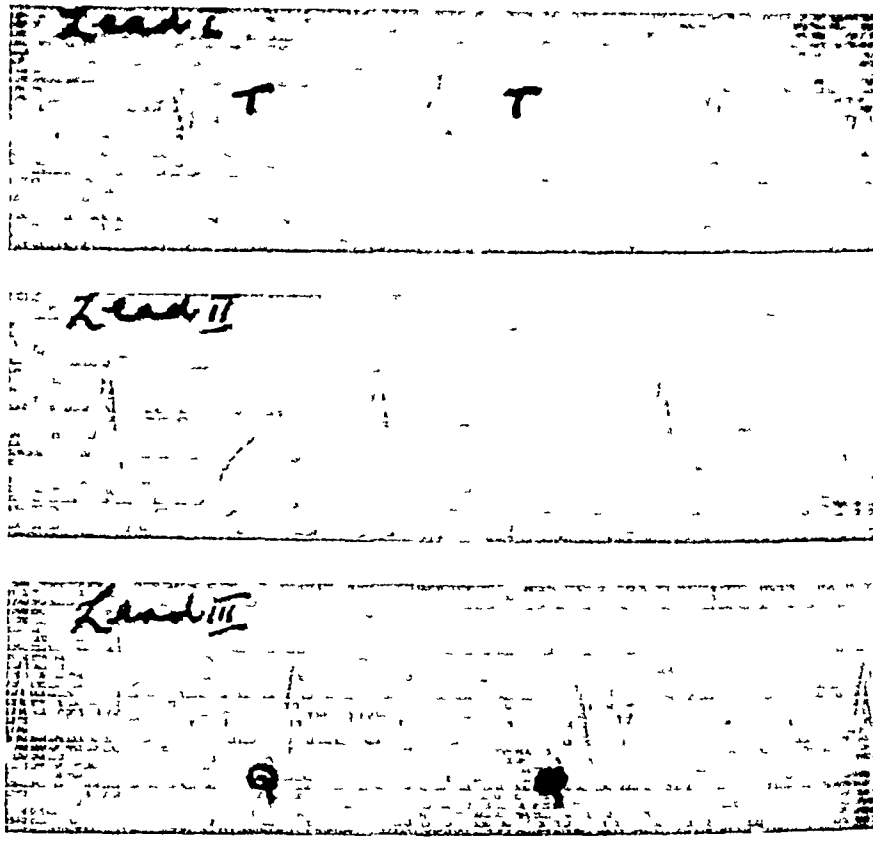
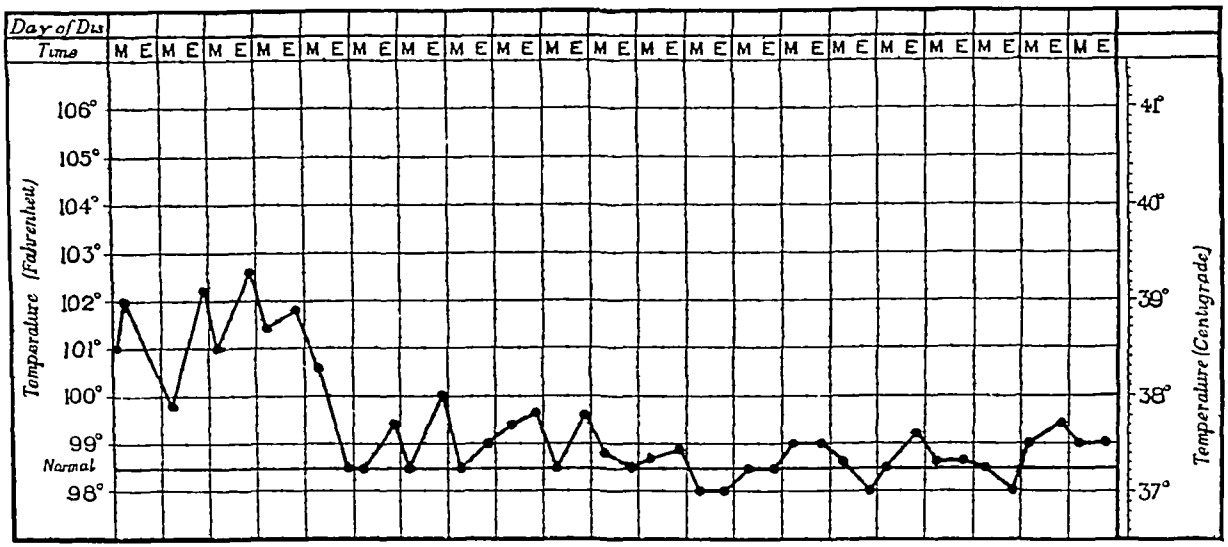


Fig 7—Patient No 9, Group I T wave small and inverted in leads I and II, absent in lead III Q wave prominent in lead III

temperature returns to normal the condition of the joints improves but they may remain more or less swollen and painful for a long time In the majority of cases the onset is gradual, there is little fever, which may last only a day or so, and hardly any articular swelling (Plate XXXVII, fig 2) The ankles, knees and wrists seemed to be the joints most commonly attacked, the next in frequency being the small joints of the hands and feets and the elbows, but almost all the joints of the body may be painful We have observed marked

PLATE XXXVII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stiffness and pain in the neck in two patients Especially in the more acute cases one or two joints are first affected, others becoming involved a day or two later The disease usually leaves behind a certain degree of stiffness due to changes in the articular and pre-articular tissues The response to salicylates is not as marked as in the case of the rheumatic fever of temperate climates In fact this drug did not seem to have any effect at all on some of the more

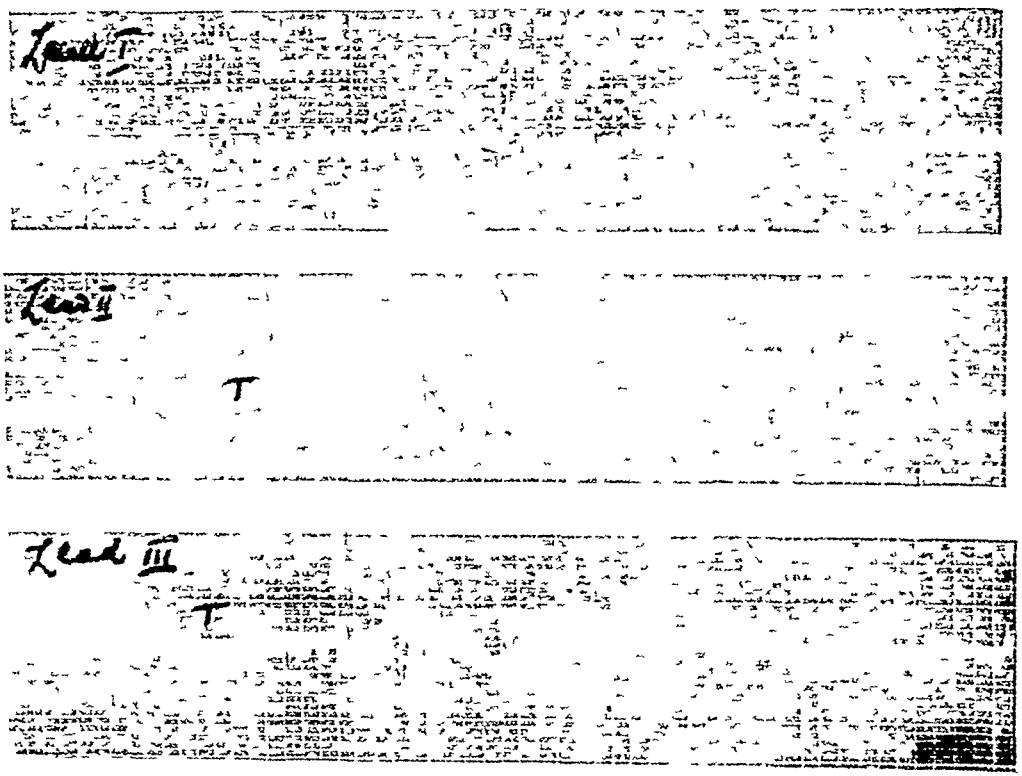

Fig 8—Patient No 1 Group II Aortic regurgitation Left sided preponderance Insertion of T wave in leads II and III R wave notched in lead III

chronic cases Rheumatic nodules were not present in any of the patients seen by us nor were there any symptoms of chorea Most of the cases of mitral disease, in whom no history of painful joints was obtained, resembled those in whom valvular lesions were associated with arthritis and were probably of the same nature In short, disease of the mitral valve in these patients would seem to be often a manifestation of an infection, generally subacute or chronic in nature, which like rheumatic fever may affect the heart or the joints or both together Whether it is actually a variety of rheumatic fever it is difficult to say but it is obviously an analogous condition It is possible that it was also the cause of the aortic disease in patients Nos 8, 13 and 19

Some explanation is needed of the fact that this condition tends to produce narrowing rather than incompetence of the mitral valve In the absence of post-mortem and bacteriological evidence it is impossible to give a definite opinion on this point Horder (1926), discussing the relation of mitral lesions to rheumatic fever, points out that while the type case of mitral regurgitation is the adult man who has had a severe but single attack of acute rheumatism in his teens, and while 'double mitral' disease is to be looked for in the child or young adult who has had repeated attacks of rheumatism, largely subacute, in many cases of mitral stenosis there is no history of rheumatism

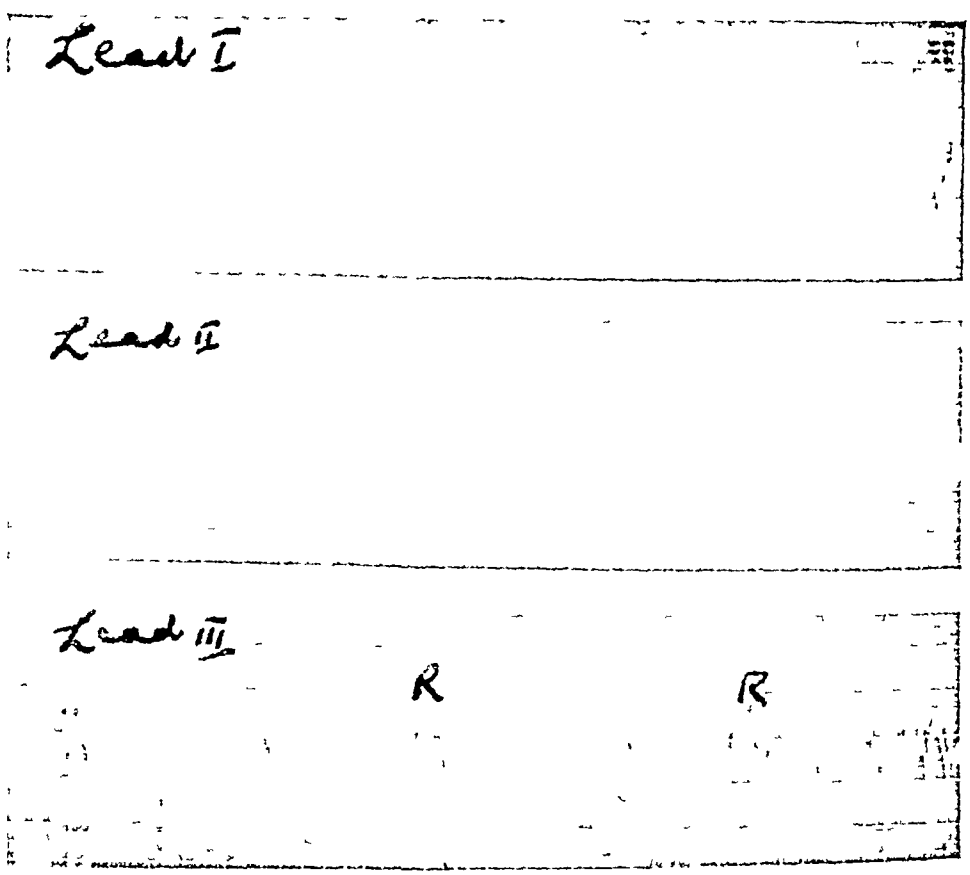

Fig 9—Patient No 3, Group II Auricular fibrillation Heart rate controlled by digitalis Right sided preponderance R wave in lead III broadened and divided

at all This is not to be taken to mean that these cases are necessarily of non-rheumatic origin, but rather that the process giving rise to stenosis is a chronic sclerosing one having none of the characters of typical rheumatic fever A slow disease process in the mitral valve analogous to that in the joints of some of our patients would be expected to lead to a gradual infiltration and fusion of the cusps and narrowing of the orifice rather than to the destructive type of lesion causing incompetence

In the second group the electrocardiograms revealed myocardial involvement in two cases In No 1 (Fig 8) the T wave was inverted in leads II and III and there was notching of the R wave in lead III In No 3 (Fig 9) in addition to evidence of auricular fibrillation there was widening and notching of the R wave in lead III

In certain cases of mitral stenosis with doubtful signs an enlarged and notched P wave, indicating auricular hypertrophy, pointed to the nature of the lesion, e g , in case No 21 (Fig 10)

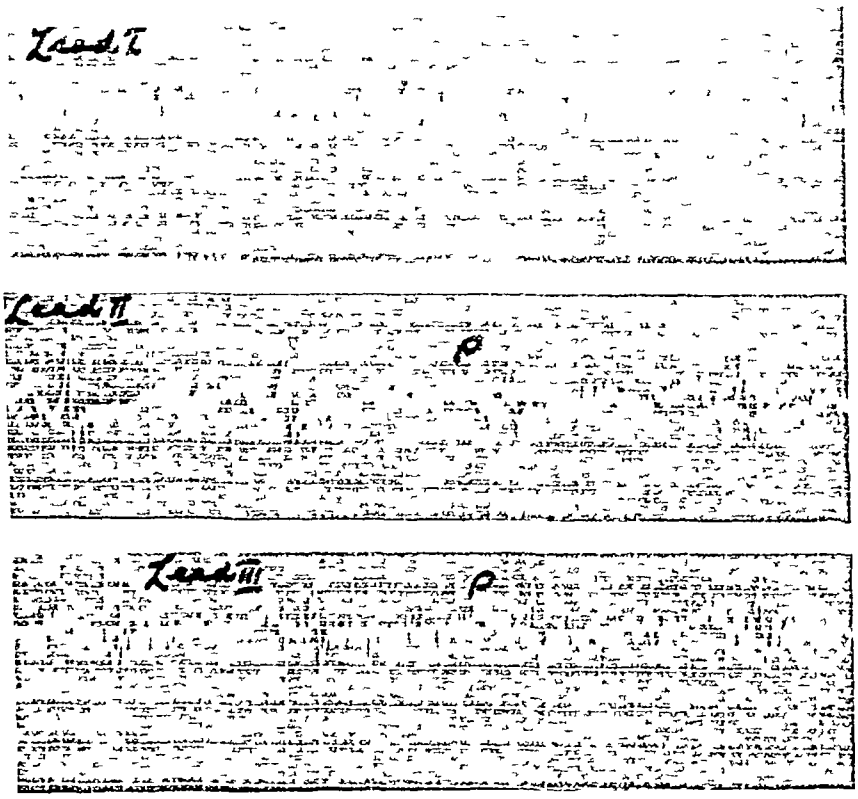

Fig 10—Patient No 21 Group II Mitral stenosis without diastolic murmur P wave enlarged and notched

Of the 6 female cases in this group three (Nos 15, 17, and 21) had pure mitral stenosis, one (No 4) pure mitral regurgitation, one (No 24) 'double mitral' disease and one (No 19) mitral stenosis and aortic regurgitation The fact that the vast majority of mitral cases were males does not admit of the conclusion that in this province mitral disease is more common among males than among females as comparatively few females seek admission to the medical wards of the hospital

SUMMARY

1 An analysis is given of 35 cases of heart disease occurring among inhabitants of the Punjab

2 In 11 patients, 9 males and 2 females, there were marked symptoms of myocardial insufficiency with no evidence of valvular disease In 7 of these including a case of auricular fibrillation the electrocardiogram gave evidence of involvement of the heart muscle

3 In 24 patients, 18 males and 6 females valvular disease was present One of these was a case of syphilitic aortic disease In all the others the mitral valve was affected One had aortic and mitral incompetence, three aortic incompetence and mitral stenosis three mitral incompetence, three (including a patient with auricular fibrillation) 'double mitral' disease and thirteen mitral stenosis

4 The importance of X-rays in the diagnosis of mitral stenosis is illustrated

5 A description is given of a form of multiple arthritis usually subacute or chronic in nature which occurs among these patients and its relation to valvular disease of the heart, especially mitral stenosis, is discussed

The electrocardiograms were taken by Dr D L Shrivastava, DSc, to whom our best thanks are due We are also indebted to Dr B S Bhandari, our House Physician, for assistance during the investigation

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LEVINE (1929) *Medicine*, VIII, p 215
STITT (1929) 'Tropical Diseases,' p 653

EXPLANATION OF PLATE XXXIX

Fig 3 Patient No 19, Group 2, mitral stenosis and aortic regurgitation Marked enlargement of left auricle

„ 4 Patient No 8, Group 2, mitral stenosis and aortic regurgitation Straight left border due to prominent pulmonary artery and enlarged left ventricle Root shadows marked

Fig 3

Fig 4

TABLE

Showing distribution of cases according to age and disease

DISEASE	TOTAL NUMBER	AGE IN YEARS						
		1—10	11—20	21—30	31—40	41—50	51—60	61—70
Group I	11		2	1	2	3	3	
Group II— Aortic regurgitation	1				1			
Aortic regurgitation and mitral regurgitation	1					1		
Aortic regurgitation and mitral stenosis	3		1	2				
Mitral stenosis	13	1	6	1	2	2	1	
'Double mitral' disease	3	1		2				
Mitral regurgitation	3			1	1		1	

SUMMARY OF CASES

Group I (No detectable valvular disease)

Case 1—I S, male, 25 Admitted on 24-8-29 with signs of congestive heart failure, viz, orthopnœa, œdema, cardiac liver, ascites and left sided pleural effusion Contracted syphilis one year ago, not treated Moderate addiction to alcohol Heart dilated Soft systolic murmur in the mitral and tricuspid areas Second aortic and pulmonary sounds weak Blood-pressure 140/115 W R strongly positive (+++) Treated specifically for syphilis and symptomatically for the cardiac condition Discharged on 3-10-29 No dropsy Liver normal size Blood-pressure 125/80 No murmur W R +— — Electrocardiogram showed left preponderance

Case 2—Fig 3 G S, male, 50 Admitted on the night of 22-10-29 with cardiac asthma This was his first attack Had fever for 4 days and cough for 5 months Contracted syphilis 15 years ago Previous to the present complaint had been in good health Catarrhal signs in both lungs Heart enlarged, impact weak, a soft systolic murmur at the apex conducted to the anterior axillary line, first sound absent Electrocardiogram indicated 'intraventricular' heart block W R negative He appeared to be making good progress but died suddenly during night of the 4th November Post-mortem Weight of heart 10 oz, fatty infiltration marked An aneurysm about the size of a tennis ball present at the apex of the left ventricle

Case 3—Fig 2 P S, male, 50 Admitted on 15-1-30 on account of attacks of præcordial pain which radiated round the chest to the left subscapular region The attacks lasted for hours—on one occasion for 24 hours During attacks the patient lay quite still and asked to have the præcordium firmly pressed The pain had no relation to meals or exertion Duration three months Previous health good W R negative Blood-pressure 195/105 on

admission when there was no pain It fell to 180/105 during an attack The pain was unaffected by nitrites There was slight hypertrophy of heart The first sound was weak and the second somewhat accentuated Electrocardiogram indicated right bundle block Treatment had no permanent effect

Case 4—K, female 17 Admitted on 23-1-30 with orthopnœa palpitation, cough, general anasarca below the level of heart, cardiac liver and ascites Symptoms were first noticed 12 years ago but the delivery of a child 3 months previous to admission seemed to have aggravated the trouble Heart moderately enlarged Apex beat diffuse heaving and appeared double No thrill Gallop rhythm at the apex A soft apical systolic murmur conducted a little to the left No accentuation of the pulmonary second sound Blood-pressure 85/50 W R negative Response to treatment poor

Case 5—Fig 1 R A male 18 Admitted with palpitation, orthopnœa, cough œdema below the level of the heart cardiac liver ascites and double pleural effusion Duration one month Had plague in 1919, after which he developed congestive heart failure and was treated in this hospital Remained fairly well until a month ago when an attack of fever lasting two days brought on the present trouble Heart moderately enlarged Apex beat diffuse and weak Auricular fibrillation No murmur Blood-pressure 105/70 W R negative Pulse about 100 per minute, quite irregular Treated with digitalis Improvement slight

Case 6—Fig 5 G S male, 35 Admitted on 18-3-30 for shortness of breath on slight exertion Duration 2 years Had syphilis 10 years ago Heart not enlarged, sounds clear, no murmur Blood-pressure 125/110 Electrocardiogram shows widening of QRS complex and inversion of T wave in lead III He left hospital on 20-3-30 before a W R could be done or a skiagram taken

Case 7—B R, male 55 Admitted on 1-10-29 with a swelling in the upper part of the left chest of 6 months' duration, cough and occasional hæmoptysis of 7 months' duration Contracted syphilis 30 years ago Swelling the size of a tennis ball with expansile pulsation and a systolic bruit Skiagram showed an aneurysm of descending arch of aorta No difference in pupils or pulse on the two sides Cough not the typical one of aneurysm Nothing abnormal in the heart on physical examination General condition poor W R ++++ Electrocardiogram showed prominent Q waves in leads II and III No response to treatment

Case 8—Fig 6 F, male, 52, a well set-up athletic individual Seen a week after a severe and prolonged attack of præcordial pain, which was most marked at the lower end of the sternum and radiated down both arms There was collapse and vomiting during the attack, which was the first of its kind Blood-pressure on examination 100/80 Heart normal in size No murmur Duplication of first sound at apex Electrocardiogram indicated coronary thrombosis

Case 9—Fig 7 M A K, male, 55 Admitted on 9-4-30, for præcordial pain on slight exertion Four years' duration Orthopnœa and œdema of legs of 5 months' duration Had had albuminuria for the last 7 years Heart moderately enlarged, apex beat diffuse and weak A soft systolic apical murmur after the first sound Cardiac liver Blood-pressure 160/110 W R negative Electrocardiogram showed widening of QRS complex and prominent Q wave in lead III No response to treatment

Case 10—Fig 4 R B, male, 50 Admitted on 22-4-30 with orthopnœa, œdema below the level of heart, ascites and cardiac liver Six months' duration Accustomed to very hard exercise—a wrestler Suffered from typhoid for 2 months 2 years ago Heart greatly enlarged Apex beat diffuse and weak A soft systolic murmur at the apex—slightly conducted to the left Blood-pressure 85 systolic Diastolic could not be taken Electrocardiogram gave evidence of 'intraventricular' block W R negative Response to treatment poor

Case 11—R B, female, aged 35 Admitted on 29-4-30 with orthopnœa, œdema below the level of heart, cardiac liver and a very troublesome cough of 3 months' duration

Confined 3 months ago Had much hæmorrhage and suffered from sepsis Cardiac symptoms developed insidiously during the puerperal fever No history of rheumatism Heart moderately enlarged A soft systolic murmur at the apex Both sounds weak Skiagram showed no encroachment upon retrocardiac space Blood-pressure 120/55 W R negative No response to treatment

Group II (Valvular disease)

Case 1—Fig 8 Aortic regurgitation T S, male, 40 Admitted on 12-2-29 with palpitation, shortness of breath and pain in the epigastrium after exertion Duration 7 months Practically bed-ridden Heart moderately enlarged Apex beat weak and diffuse Both sounds replaced by soft murmurs, systolic and diastolic, best heard in the aortic area but audible over the whole of the præcordium and a little beyond it on all sides Loud systolic sounds audible over the big arteries Blood-pressure 180/80 Liver not enlarged W R +++ Electrocardiogram showed left sided preponderance and inversion of T wave in leads II and III Did not improve

Case 2—Mitral stenosis A M, male, 18 Admitted on 3-10-29 with palpitation and shortness of breath on exertion Six months' duration No history of rheumatism Heart moderately enlarged No thrill First mitral sound sharp and short, second pulmonary sound accentuated An early diastolic, a rough presystolic and a soft systolic murmur (just after the first sound) audible at the apex Catarrhal signs in lungs Repeated examination of sputum for tubercle bacilli negative All murmurs disappeared when patient got a wave of temperature Response to treatment poor

Case 3—Fig 9 'Double mitral' disease Admitted on 24-10-29 with orthopnœa, palpitation, cough and œdema Symptoms first noticed 2 years ago Joint 'rheumatism' 5 years ago Heart enormously enlarged pushing a big cardiac liver bodily downwards A systolic and diastolic thrill present over the lower part of præcordium Auricular fibrillation A diastolic and a soft systolic murmur at the apex—the latter well conducted to the left Second pulmonary sound accentuated Catarrhal signs in lungs Electrocardiogram showed myocardial involvement Response to treatment poor

Case 4—Mitral regurgitation J, female, 20 Admitted on 5-11-29 with shortness of breath and palpitation on exertion, low fever and cough About 3 years' duration Miscarried 3 years ago Had moderate hæmoptysis lasting 4 days in April 1929 Occasional œdema of the legs Heart moderately enlarged, impact forcible and diffuse, no thrill A short systolic apical murmur conducted to the left armpit and inferior angle of scapula, audible over the whole of præcordium with slightly diminished intensity Second pulmonary sound accentuated Cardiac liver Blood-pressure 150/50 W R negative Treated symptomatically Response to treatment good

Case 5—Mitral regurgitation M D, male, 38 years Admitted on 3-12-29 with palpitation and shortness of breath on exertion One year's duration Had joint 'rheumatism' lasting 2 months in 1913 Heart moderately enlarged Veins in neck prominent Epigastric pulsation marked Apex beat diffuse and forcible A blowing systolic murmur at the apex conducted to the inferior angle of left scapula and audible with diminishing intensity over the whole of præcordium Second pulmonary sound accentuated Blood-pressure 160/90 W R negative Liver pulsating and big Treated symptomatically Much improved

Case 6—Mitral regurgitation A R, male, 60 Admitted on 3-12-29 with orthopnœa and œdema below the level of heart Three months' duration History of primary sore 12 years ago No history of 'rheumatism' Heart much enlarged Apex beat diffuse and forcible A systolic murmur at the apex conducted to the inferior angle of left scapula and audible over the whole of præcordium—musical at the apex, elsewhere *soft* and blowing Second pulmonary sound accentuated Cardiac liver Blood-pressure 135/88 W R negative No response to ordinary measures Improved much with novasurol Discharged practically free from symptoms

Case 7—'Double mitral' disease M S, male aged 26 Admitted on 21-1-30 with orthopnœa, ascites cough and palpitation Symptoms first noticed 2 years ago History of joint 'rheumatism' lasting 3 months, 9 months ago Cyanosis moderate Veins in neck full and liver enlarged Signs of congestion in both lungs Heart much enlarged Apical presystolic and systolic murmurs present accompanied by thrills The systolic conducted to inferior angle of left scapula First mitral sound sharp and short, second pulmonary sound accentuated Routine treatment Discharged moderately improved

Case 8—Plate XXXVIII, fig 2 Mitral stenosis and aortic regurgitation A M, male, 30 Admitted on 25-1-30 with palpitation and shortness of breath on exertion Eight years' duration Cough for 2 months History of joint 'rheumatism' 10 years ago Heart moderately enlarged Diastolic thrill at apex Apical diastolic murmur with a presystolic accentuation leading up to a short sharp first sound, also a soft apical systolic murmur following first sound, conducted a small distance to the left An aortic regurgitant murmur with maximum intensity to left of sternum opposite third chondrosternal junction, soft and blowing in character and conducted a little below the nipple Second pulmonary sound accentuated No sounds over arteries Liver slightly enlarged Blood-pressure 110/60 W R negative Did not improve

Case 9—Mitral stenosis T D male, 19 Admitted on 1-2-30 with lobar pneumonia Heart slightly enlarged A soft systolic murmur with maximum intensity over the left half of sternum opposite the 2nd and 3rd interspaces completely replacing the 1st sound and conducted more towards the base than the apex Second pulmonary sound accentuated No signs of failing compensation No history of 'rheumatism' X-ray evidence of left auricular enlargement

Case 10—Aortic regurgitation and mitral regurgitation K C, male, 41 Admitted on 8-2-30 with orthopnœa, œdema, cough and palpitation About 2 months' duration Chronic alcoholic Had glycosuria 2½ years ago which yielded to dietetic measures Heart much enlarged Apex beat diffuse and weak A musical systolic murmur, best heard at the apex but audible over the whole of the præcordium and conducted beyond the apex A soft diastolic murmur best heard over the sternum opposite the 3rd costosternal junction, conducted to a little beyond the apex Loud systolic sounds over the big arteries Blood-pressure 134/60 Cardiac liver W R ++++ Died on 12-2-30

Case 11—Mitral stenosis M S, male, 32 Admitted on 13-2-30 with palpitation and breathlessness on exertion Two years' duration Occasional œdema of legs after exertion One year's duration History of joint 'rheumatism' 4 years ago Marked anæmia due to ankylostomiasis Heart moderately enlarged Apex beat diffuse First mitral sound sharp and short followed by a soft systolic murmur best heard inside the apex but audible near the base as well—not conducted outside the præcordium Two months later a presystolic apical murmur detected after exertion Second pulmonary sound accentuated Blood-pressure 110/55 W R negative *X*-ray evidence of enlargement of the auricles

Case 12—Mitral stenosis G, male, 8 Attended hospital on 17-2-30 with shortness of breath and palpitation on slight exertion Duration 2 months History of 'rheumatism' 3 months previously Heart moderately enlarged A rather rough systolic murmur at the apex and just outside it First sound sharp and short Second pulmonary sound accentuated Liver enlarged

Case 13—Mitral stenosis and aortic regurgitation R R, male, 20 Admitted on 18-2-30 with pyelitis Heart condition detected on routine examination No cardiac symptoms History of joint 'rheumatism' 4 years ago Heart slightly enlarged A diastolic thrill at the apex A soft diastolic murmur best heard over the sternum opposite the 3rd chondrosternal junction and conducted towards the apex A rumbling diastolic murmur at the apex Systolic sounds over the femoral and brachial arteries in the erect position Blood-pressure 110/35 Liver not enlarged W R negative

Case 14—Plate XXXVIII, fig 1 Mitral stenosis S R male 42 Admitted on 25-2-30 with joint 'rheumatism' of about 6 months' duration A soft systolic murmur confined to the apex, replacing the 1st sound Second pulmonary sound accentuated X-ray evidence of enlarged left auricle Two months later a slight presystolic murmur could be detected after exertion

Case 15—Mitral stenosis S female, 32 Admitted on 25-2-30 with joint 'rheumatism' of about one year's duration with periods of remission A soft systolic murmur, replacing the 1st sound audible at the apex with the maximum intensity just to the left of sternum opposite the 3rd and 4th costal cartilages The second sound at the mitral and the pulmonary area accentuated Nearly one month later a soft early diastolic apical murmur developed

Case 16—Mitral stenosis S, male, 13 Admitted on 27-2-30 with cough, pain in the side and blood-streaked sputum Heart condition detected on routine examination History of 'rheumatism' 5 months ago Heart moderately enlarged An early diastolic and presystolic thrill present at the apex First mitral sound sharp and short, second pulmonary sound accentuated At the apex a rough rumbling murmur occupying the whole of diastole with presystolic accentuation Liver 2 fingers enlarged Lungs congested Response to treatment good

Case 17—Mitral stenosis W B, female, 25 Admitted on 8-3-30 Had painful joints off and on for 8 years Heart condition detected on routine examination Heart moderately enlarged No thrill Apical first sound sharp and short, preceded by a presystolic and followed by a soft systolic murmur Also an early diastolic murmur present at the apex Second pulmonary sound accentuated No signs of failing compensation

Case 18—Mitral stenosis J R, male, 12 Attended hospital on 8-3-30 with palpitation and shortness of breath on exertion About 6 months' duration History of joint 'rheumatism' 2 years ago Heart slightly enlarged No thrill First sound sharp and short at the apex, second pulmonary sound accentuated A soft apical systolic murmur conducted a little to the left following the first sound Liver 2 fingers enlarged

Case 19—Plate XXXIX, fig 3 Mitral stenosis and aortic regurgitation S, female, 30 Admitted on 18-3-30 with breathlessness and palpitation on exertion, œdema of legs, cough and pain in the abdomen (perihepatitis) No history of 'rheumatism' Heart moderately enlarged Apex beat diffuse Three murmurs (1) a soft systolic localized at apex, (2) a rough rumbling diastolic to the left of sternum, in the 3rd and 4th interspaces, (3) a soft blowing diastolic in the aortic area Blood-pressure 85/60 Cardiac liver W R negative Slight improvement

Case 20—Mitral stenosis H B, male, 18 Admitted on 3-4-30 with palpitation and breathlessness on exertion of 2 years' duration History of joint 'rheumatism' 4 years ago Heart moderately enlarged Apex beat diffuse Early diastolic and presystolic thrills at the apex A rough rumbling apical diastolic murmur with presystolic accentuation First mitral sound sharp and short Second pulmonary sound markedly accentuated A soft systolic murmur occupying the short pause heard slightly inside the apex Cardiac liver Blood-pressure 105/65 W R negative

Case 21—Fig 10 Mitral stenosis R B, female, 60 Admitted on 15-4-30 with painful swelling of the joints Had 'rheumatism' off and on for 6 years First mitral sound roughened and followed by a localized soft systolic murmur Heart not enlarged Blood-pressure 95/70 W R negative Electrocardiogram shows an enlarged and notched P wave

Case 22—Mitral stenosis S R, male, 46 Admitted on 17-4-30 with chronic malaria Heart condition detected on routine examination Heart slightly enlarged Apex beat diffuse A soft systolic murmur best heard just inside the apex but also audible over the whole of the left half of the præcordium First mitral sound sharp and short, second pulmonary sound accentuated A presystolic murmur detected after exertion Blood-pressure 110/55 W R negative No history of rheumatism

Case 23.—Mitral stenosis. M. K., male, 20. Admitted on 21-4-30 with acute bronchitis. History of two attacks of 'rheumatism,' one about 15 years ago, the other about 2 years ago. Heart moderately enlarged. Apex beat diffuse. An early diastolic and presystolic thrill at the apex. Apical diastolic murmur with presystolic accentuation. First mitral sound sharp and short. Second pulmonary sound accentuated. Blood-pressure 100/75. W. R. negative.

Case 24.— Double mitral disease. K. B., female, 7. Seen on 26-4-30 with joint pains of 3 months' duration, palpitation, precordial uneasiness and occasional fever and sweating. The onset of joint pains was associated with mild pyrexia. Heart moderately enlarged. A faint systolic thrill at the apex. Apex beat diffuse. A musical systolic apical murmur conducted to the inferior angle of left scapula. First mitral sound sharp and short. Pulmonary second sound reduplicated. Blood-pressure 100/60. Liver enlarged.

STUDIES ON THE ENLARGED MALARIAL SPLEEN

Part III

FURTHER OBSERVATIONS ON THE EFFECTS OF QUININE AND ALKALIS ON THE BLOOD PICTURE, TOGETHER WITH SOME REMARKS ON THE NATURE OF THE ENLARGED MALARIAL SPLEEN

BY

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EXPERIMENTS recorded in a previous paper (Hughes and Shrivastava, 1929) showed that in man quinine in anti-malarial doses produced changes in the blood picture indicative of splenic contraction. After an intravenous injection there was usually a sharp rise in the white cells which returned to the pre-injection level or below it in less than two hours. During oral administration a small gradual increase in leucocytes occurred. The effect of oral quinine, however, was found to be greatly increased when alkalis were given in quantities sufficient to render the urine alkaline.

The present communication deals with further experiments undertaken with the object of determining the manner in which alkalis affect the action of quinine on the spleen and of ascertaining whether oral quinine and alkalis cause increased proliferation of leucocytes as well as expulsion of cells from the spleen into the circulating blood. The observations were made mostly on individuals whose spleens were enlarged from chronic malaria but who showed no signs of active disease. The methods employed were those already described (Hughes and Shrivastava, 1929). Three sets of experiments were carried out. In one set the spleen was made to contract by injecting adrenalin subcutaneously (1 c.c. of 1 in 1,000 solution) and the increase in leucocytes compared with that produced in the same subject by injection of adrenalin after alkalization. In

another set the effect of alkalis on the leucocytosis resulting from intravenous quinine was studied In the third the changes produced in the leucocyte picture by oral quinine and alkalies were observed over a period of several days Variations in the red cell count were not noted as it had been found that hæmolysis followed intravenous injections of quinine in some malarial subjects

(a) *Effect of alkalies on the leucocytosis produced by adrenalin*

The results of some experiments are given in Table I It is seen that in every case the effect of adrenalin like that of oral quinine was increased by

TABLE I

Showing the effect of alkalies on the leucocytosis caused by the injection of adrenalin

No	Date	Name	Clinical notes	W B C per c.mm		Remarks
				Before injection	After injection *	
1	3-12-29	B R	Convalescent pneumonia (12 days) Liver slightly enlarged Spleen about 1" below the costal margin	11 070	14,030 (+26·7)	
	5-12-29			11 070	22 600 (+104·2)	Urine alkaline
2	18-12-29	N	Convalescent pneumonia Liver enlarged about 1" and spleen about 2½" below the costal margin History of malaria	9 230	11 370 (+23·2)	
	19-12-29			8 130	11,270 (+38·6)	Urine alkaline
3	23-12-29	U S	History of malaria Liver not enlarged Spleen enlarged up to umbilicus	7,630	10,570 (+51·6)	
	26-12-29			6 870	11 500 (+67·1)	Urine alkaline
4	9-1-30	H A	History of malaria Spleen filling up almost the whole of the abdominal cavity, extending up to the right iliac fossa	9 630	13,600 (+41·0)	
	10-1-30			9 200	17,830 (+93·8)	Urine alkaline

* Figures in brackets indicate percentage changes

giving alkalis and that the increase varied in degree As the smooth muscle of the spleen, like smooth muscle in general, is rendered more sensitive to adrenalin stimulation by increase of pH, it is possible that the variations observed were due to some extent to the degree to which the reaction of the blood was altered This would be expected to vary in different individuals as it depends not only on the initial pH of the blood and the production of acid substances but also on the delicacy of the acid-base regulating mechanism It is probable, however, that the condition of the spleen was also responsible for these variations Pathological changes alter the contractility of the organ and a greater proportion of the total possible contraction may have been produced by adrenalin alone in some cases than in others When the spleen is very fibrous little or no contraction is possible

(*b*) *Effect of alkalis on the leucocytosis produced by intravenous quinine*

The rise in leucocytes produced by therapeutic doses of quinine given by the intravenous route was not increased by giving alkalis (Table II) This would indicate that the quinine alone caused maximal contraction of the spleen Generally speaking intravenous administration of quinine in malaria does not

TABLE II

Showing the effect of alkalis on the leucocytosis caused by intravenous injection of quinine (6 *gr*)

No	Date	Name	Clinical notes	W B C per c mm		Remarks
				Before injection	After injection *	
1	5-11-29	M A	Fever off and on for the last 7 years Spleen enlarged to umbilicus	7,130	8,870 (+24 4)	
	8-11-29			8,570	9,600 (+12·0)	Urine alkaline
2	11-11-29	A	Occasional attacks of fever for sometime past Moderate enlargement of spleen	10,570	13,430 (+27 1)	
	13-11-29			11,300	13,300 (+17 7)	Urine alkaline
3	19-11-29	G	Chronic malaria Spleen filling almost the whole of the abdominal cavity Liver also enlarged	5,770	7,300 (+26 5)	
	21-11-29			5,430	6,870 (+26 5)	Urine alkaline

* Figures in brackets indicate percentage changes

appear to possess any advantages over oral administration with regard to its ultimate therapeutic action, but Acton *et al* (1921) found that in benign tertian infections it caused the parasites to disappear more rapidly from the peripheral blood This effect may be connected with the rapid mobilization of monocytes produced in the spleen, as the drug itself rapidly leaves the blood Hattman and Zilva (1918) found that after an intravenous dose of 0 6 g , 90 per cent had left the blood in twenty minutes Intravenous quinine produced no leucocytosis in patients with indurated fibrous spleens

(c) Observations on the leucocytosis produced by oral quinine and alkalis

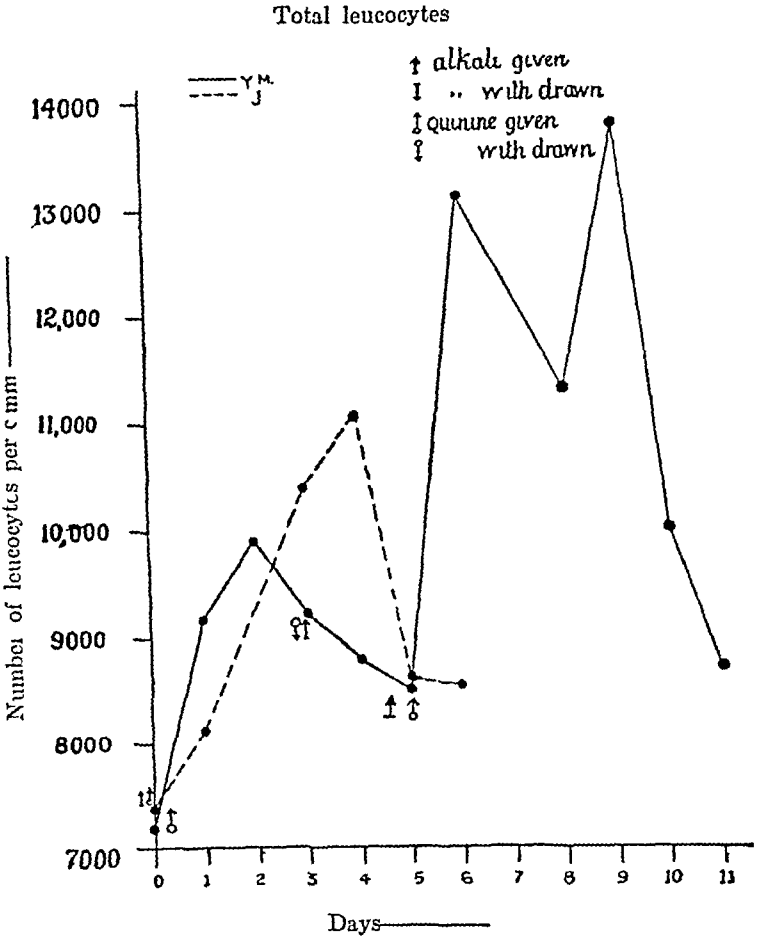
The experiments recorded above (*a*) would lead one to conclude that alkalis accentuate the action of oral quinine on the spleen by rendering the contractile tissues more sensitive to stimulation by the alkaloid While a sudden rise in leucocytes would be produced by this effect it is also possible that increased proliferation of white cells, especially monocytes, might be brought about by this combination of drugs or by quinine alone Giemsa (1927) is of opinion that quinine is taken up by the cells of the reticulo-endothelial system and if this is so a continuous stimulus to the proliferation of these cells would be provided during the absorption of quinine from the alimentary canal With a view to obtaining information on this point differential leucocyte counts were made for several days in individuals receiving quinine and alkalis by the mouth Some typical results are given in Table III, and two experiments are represented graphically in the following figure (*see* p 505)

In general it was found that the rapid rise in the total white cells began in a day or so and persisted for 2 to 4 days There was then a sudden fall to about the original level A prolonged monocytosis was not produced It was noticed that the reduction in size of the spleen corresponded to the increase in leucocytes, a rapid reduction occurring during the period of maximal leucocytosis and a much slower reduction afterwards When no increase in the white cells was produced there was no noticeable effect on the size of the splenic tumour This was the case in patient 4 in whom there was actually a fall in leucocytes and whose spleen was very hard on palpation These facts point to the conclusion that, at least in the absence of malarial infection, the leucocytosis brought about by quinine or by quinine and alkalis is entirely, or almost entirely, due to contraction of the spleen

(d) Some remarks on the nature and significance of the enlarged spleen in chronic malaria

The results so far obtained permit of certain conclusions regarding the nature and significance of splenic enlargement in prolonged or repeated malarial infection It has been shown (Hughes and Shrivastava, 1929), that contraction

by adrenalin of the enlarged spleen in chronic malarial subjects often causes a much smaller rise in the red blood cells than contraction of a normal spleen, although a degree of leucocytosis much greater than normal may be produced



In many cases monocytes took a very prominent part in the increase in white cells This occurred in patients who were, or had recently been, suffering from slight active manifestations of the disease in the form of irregular fever In two patients previously referred to (Hughes and Shrivastava, 1929) in whom the disease was getting the upper hand the spleen expelled no monocytes on contraction, but, when the disease had been got under control by means of quinine, splenic contraction gave rise to a marked increase of monocytes in the peripheral blood These facts indicate that proliferation of the reticulo-endothelial cells is the immunity reaction of the tissues to malarial infection just as proliferation of the neutrophile granulocytes is the cellular reaction to pneumococcal infection and 'local infiltration of plasma cells and lymphocytes represents the processes of a local tissue immunity' in syphilis (Warthin, 1929)

A spleen enlarged because of great proliferation of the reticulo-endothelial cells

may therefore be looked upon as a defence against malaria Its capacity to store red blood corpuscles may however, be diminished owing to invasion of the blood spaces by the splenic cells

In some individuals (e g, Nos 1 and 3, Table III) with enlarged malarial spleens monocytes were not expelled into the blood in large numbers when the spleen was made to contract although a leucocytosis did occur In these patients there had been no fever for a long period and there were no parasites in the peripheral blood There had, therefore, been no stimulus to reticulo-endothelial hypertrophy for some time It is probable that the spleens although still able to contract, contained large amounts of fibrous tissue as prolonged treatment did not effect much reduction in size Histological examination shows that more or less fibrosis always accompanies proliferation of the splenic reticulo-endothelial cells in chronic malaria In long standing cases (e g, patient 4, Table III) this process may be so marked that contractility is practically abolished

Splenomegaly in chronic malaria may, therefore, be due mainly to proliferation of the reticulo-endothelial cells or to fibrosis or to both In the first case a diminution in size of the organ will result from removal of the stimulus to cell proliferation, i e, by killing off the malarial parasites When, however, overgrowth of fibrous tissue is largely responsible for splenic enlargement this will remain permanently to a greater or less extent unless shrinkage of the fibrous tissue occurs Sinton (1929) noticed that reduction in size of enlarged malarial spleens when oral quinine and alkalis were given was at first rapid and then gradually slowed down From the results of our observations it would appear that the initial rapidity of effect is here due to contraction of the smooth muscle of the spleen In the causation of the splenic tumour that occurs in an acute attack of malaria engorgement with blood plays an important part, but here also there is in ordinary cases proliferation of the reticulo-endothelial cells as such cells usually appear in the blood in increased numbers when the fever disappears

Changes analogous to those occurring in the spleen are also seen in the portal spaces of the liver in chronic malaria These changes often result in hepatic enlargement which may disappear when the parasites are killed off They may, however, lead to portal obstruction from overgrowth and contraction of fibrous tissue

Conclusions

1 Adrenalin produces a greater contraction of the spleen when large doses of alkalis are given than when alkalis are withheld

2 Alkalis do not increase the contraction of the spleen brought about by intravenous injections of quinine in anti-malarial doses

3 During administration of oral quinine and alkalis there is a prompt rise in the white cell count which, however, falls to about the original level after 2 to 4 days There is no evidence of increased cell proliferation

TABLE III

Showing the effect of oral quinine and alkalis on the blood picture

No	DATE	NAME	CLINICAL NOTES	W B C PER C MM	DIFFERENTIAL COUNT PER C MM					REMARKS
					Large lympho-cytes	Small lympho-cytes	Mono-cytes	Poly-nuclears	Eosino-phils	
1	21-11-29	G R	Chronic malaria with asthma Liver and spleen slightly enlarged No fever for a long time	11,770	1,024	1,836	1,460	3,096	4,355	40 grs of quinine daily from 21-11-29
	23-11-29			13,400	1,568	2,050	1,876	3,565	4,341	Quinine stopped and alkalis given on 23-11-29
	28-11-29			11,630	1,861	1,628	1,279	2,988	3,873	Urine alkaline Alkalis + quinine started on 28-11-29
	29-11-29			14,700	1,911	2,263	1,676	3,233	5,630	
2	8-1-30	Y M	Used to get attacks of malaria during the 'malaria season' Spleen about 5" and liver 3" below costal margin	7,700	1,109	1,286	893	4,058	354	40 grs of quinine daily from 8-1-30
	9-1-30			9,170	945	1,926	1,440	4,521	339	
	10-1-30			9,320	1,023	1,559	1,063	5,987	298	
	11-1-30			9,930	558	1,015	1,237	5,842	277	Quinine stopped on 11-1-30 and alkalis given
	12-1-30			8,800	1,232	2,376	819	4,110	264	Urine alkaline
	13-1-30			8,500	1,393	2,124	791	3,715	476	„ „
	14-1-30			13,130	919	2,009	1,142	8,798	263	Alkalis + quinine from 13-1-30
	16-1-30			11,330	453	941	839	8,804	295	
	17-1-30			13,830	1,148	1,660	1,757	8,853	415	
	18-1-30			10,030	1,374	1,705	1,204	5,417	331	
	19-1-30			8,700	696	1,453	896	5,333	322	

TABLE III—*concld*

No	Date	Name	Clinical notes	W B C per c mm	Differential count per c mm					Remarks
					Large lympho-cytes	Small lympho-cytes	Mono-cytes	Poly-nucleais	Eosino-phils	
3	17-1-30	J	'Used to get occasional attacks of fever' Liver and spleen moderately enlarged for the last 4 years	7,400	910	1,872	1,332	3,085	170	Alkalis + quinine (10 grs daily) from 17-1-30
	18-1-30			8,130	1,220	1,845	1,488	3,439	138	
	20-1-30			10,400	1,217	3,598	1,602	3,775	208	
	21-1-30			11,100	666	2,586	1,487	5,961	400	
	22-1-30			8,070	646	1,291	920	1,922	291	
	23-1-30			8,030	1,068	1,397	1,261	3,902	102	
4	23-1-30	H A	Old history of chronic malaria Spleen filling up almost the whole of the abdominal cavity, extending up to right iliac fossa	8,730	786	1,309	1,222	1,513	899	Normal
	24-1-30			9,030	876	2,077	930	1,361	786	Alkalis on 23rd urine alkaline on 21-1-30
	25-1-30			8,830	915	1,590	1,171	1,265	857	Alkalis and quinine from 21-1-30
	27-1-30			7,770	909	1,131	956	3,807	963	
	28-1-30			7,230	701	1,168	889	3,498	672	
	30-1-30			7,770	777	1,888	723	3,781	598	

4 When the spleen is fibrous and indurated the leucocytic response to both adrenalin and quinine is greatly diminished or abolished

5 The significance of the enlarged malarial spleen is discussed

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STUDIES ON THE ENLARGED MALARIAL SPLEEN

Part IV.

EFFECT OF ORAL ADMINISTRATION OF PLASMOQUINE ON THE BLOOD PICTURE

BY

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PLASMOQUINE, a synthetic alkyl-amino-6-methoxy-quinoline salt, was introduced by German chemists in 1925, for the treatment of malaria. It is structurally related to quinine in that it contains the quinoline nucleus but the piperidine nucleus which is contained in the quinine molecule does not enter into its composition. Therapeutically it differs from quinine chiefly in its action on the subtertian malarial parasite. Quinine has a strong lethal action on the asexual forms of this organism and hardly any effect on the sexual forms or crescents. Plasmoquine, on the other hand, acts much more strongly on the crescents than on the non-sexual forms. In view of the relations of plasmoquine to quinine and of the fact that the latter drug in anti-malarial doses causes contraction of the spleen, experiments on the lines already described (Hughes and Shrivastava, 1929) were carried out to determine whether plasmoquine brings about splenic contraction or in any other way alters the blood picture.

METHOD

Plasmoquine in doses of 0·02 g. was given by the mouth five times a day and blood counts were made at the same time each morning before the subjects received any food. Observations lasted 4 to 6 days. Most of the patients had enlarged malarial spleens but none had any signs of active disease. In some cases the urine was kept alkaline, as this procedure was found markedly to enhance the effect of quinine on the spleen.

RESULTS

Typical results are given in Table I. There was usually a diminution in the red cells but occasionally an increase was seen (Case 2). As a rule the

TABLE I

Showing the effect of plasmoquine on the blood picture

No	Date	Name	Clinical notes	R B C per c mm (millions)	W B C per c mm	Differential count per c mm					Remarks
						Large lymphocytes	Small lymphocytes	Monocytes	Polymuclears	Eosinophiles	
1	29-1-30	S	Convalescent broncho-pneumonia Spleen and liver not palpable	4 22	10,900	905	2 801	1,308	5,809	76	Plasmoquine 0·1 g per day from 29-1-30
	30-1-30			4 01	11,570	1,770	3,518	691	5,552	35	
	31-1-30			3·07	10,770	1,185	3,091	722	5 709	65	
	1-2-30			4 18	9,370	815	2,436	337	5 687	91	
	3-2-30			3 79	9,000	990	2,916	321	1 712	27	
	4-2-30			3·92	9,630	1,223	2,215	703	5 325	161	
	5-2-30			4 02	9,870	760	2,990	523	5 158	138	
	6-2-30			4 21	9,430	566	2,707	377	5,658	123	
2	2-2-30	M S	Convalescent lobar pneumonia History of malaria Ankylostomiasis Spleen enlarged about 1½" below the costal margin	4 53	11,070	808	1,661	551	7 382	661	Plasmoquine 0·1 g per day from 2-2-30
	3-2-30			4·77	10,100	1,010	1,959	636	5,828	636	
	4-2-30			5 45	9,800	784	1,960	715	5,723	617	
	5-2-30			5 26	9,470	758	1,260	691	5,965	795	
	6-2-30			5 05	8,330	808	1,333	525	1,831	833	
3	7-2-30	H A	History of malaria Spleen filled greater part of the abdominal cavity—extended up to the right iliac fossa Liver not enlarged Ankylostomiasis	4 74	9,270	899	1,631	1,177	4,598	961	Plasmoquine 0·1 g per day from 7-2-30
	8-2-30			4 76	9,330	961	2,332	746	4,114	877	
	10-2-30			4 39	8,330	916	2,166	641	3,941	666	
	11-2-30			4 03	8,200	738	1,968	574	4,370	519	
	12-2-30			3 97	8,470	788	1,863	652	4,514	652	

1	14-2-30	B	Chronic malaria Irregular enlargement of the left lobe of the liver extending about 3½" below the xiphisternum Spleen enlarged about 2½" below costal margin Ankylostomiasis	6·76	8,170	793	1,928	629	4,436	384	Urine alkaline
	15-2-30			6·81	8,000	504	1,256	720	5,176	344	Alkalis + plasmoquine 0·1 g per day from 14-2-30
	17-2-30			5·76	8,430	565	1,290	700	5,454	422	
	18-2-30			5·91	7,230	528	1,207	651	4,266	578	
	19-2-30			6·16	7,100	873	781	689	4,190	568	
5	18-2-30	J	History of malaria Liver and spleen moderately enlarged for the last 4 years	6·24	8,570	746	1,199	1,260	4,542	823	Plasmoquine 0·1 g per day from 18-2-30
	19-2-30			6·27	8,570	369	1,654	600	5,888	60	
	20-2-30			5·35	7,200	670	1,563	598	4,298	72	
	21-2-30			5·45	7,500	750	1,777	698	4,253	23	
	22-2-30			5·43	7,400	540	1,702	622	4,410	126	Plasmoquine stopped on 22-2-30
	5-3-30			6·31	7,200	1,317	1,872	720	2,880	410	
	6-3-30			6·11	8,330	1,474	1,524	833	3,997	500	Urine alkaline
	7-3-30			5·98	7,030	795	2,482	731	2,932	91	Plasmoquine 0·1 g per day + alkalis from 6-3-30
	8-3-30			5·53	6,730	942	1,864	511	3,391	20	
	10-3-30			5·73	7,270	1,113	1,868	749	3,467	73	
	11-3-30			6·16	7,730	982	1,700	696	4,275	77	
6	25-2-30	M S	Ankylostomiasis Mitral disease Chronic malaria Spleen palpable about 1" below the costal margin	3·39	6,470	1,663	751	414	3,493	149	Urine alkaline Plasmoquine 0·1 g a day + alkalis from 27-2-30
	27-2-30			3·29	6,330	1,158	975	481	3,311	405	
	28-2-30			3·14	6,170	450	2,184	389	2,857	290	
	1-3-30			3·23	5,630	675	1,784	242	2,607	321	
7	2-4-30	A D	Occasional attacks of malaria for the last 5 months Liver slightly enlarged Spleen enlarged 3" below costal margin	4·05	6,830	567	1,523	232	4,255	253	Plasmoquine 0·1 g from 2-4-30
	3-4-30			3·97	6,430	1,074	1,607	514	3,067	167	
	5-4-30			3·74	5,770	675	1,229	394	3,290	173	
	7-4-30			4·00	5,300	652	1,256	456	2,687	249	

total white cell count also decreased There was generally a rise in the lymphocytes, especially the small variety, during the first two or three days When the monocytes were above or at the upper limit of normal a sharp fall occurred at the beginning of the experiment but when they were comparatively few in number only small irregular changes were seen The variations in the polynuclears and eosinophiles were inconstant In one patient (No 5) there was a marked fall in the latter cells in two experiments In individuals who were receiving alkalis the alterations in the leucocyte picture were sometimes accentuated but not to a marked degree In some patients the drug gave rise to headache and pains in the stomach but cyanosis was not produced in any subject

The results show that plasmoquine, unlike quinine, does not cause contraction of the spleen It is true that in some of the patients the spleen may have been so indurated that very little contraction was possible, but this was not the case in the majority including those with no splenomegaly The changes in the differential count do not permit of much discussion The fall in monocytes when these cells were numerous might indicate that in some way or other they were retained in the spleen The temporary increase in lymphocytes suggests the expulsion of a reserve of these cells into the circulating blood It is interesting to note that changes in the differential leucocyte count in some respects analogous to those described above were found to occur after injection of 1 c c of adrenalin (1 in 1,000) in a healthy subject whose spleen had been removed a year previously on account of traumatic rupture (Table II)

TABLE II

Changes in the blood picture of a healthy splenectomized subject after 1 *c c adrenalin* (1 *in* 1,000)

	Before	After (20 mins)
Red cells	5 50 (millions)	5 61 (millions)
Total white cells	8,100	8,330
Large lymph	1,191	1,191
Small „	2,293	3,032
Monocytes	834	633
Polynuclears	3,540	3,082
Eosinophiles	243	391

CONCLUSIONS

1 Plasmoquine given by the mouth for 4 to 6 days in daily doses of 0 1 g caused no contraction of the spleen

2 The most constant changes produced in the blood picture were —

(*a*) A fall in the red cells

(*b*) A fall in the total white cells

(*c*) A temporary rise in the lymphocytes

(*d*) A sharp fall in the monocytes when these cells were at or above the upper limit of normal

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FURTHER OBSERVATIONS ON THE SERUM CALCIUM AND PLASMA CHOLESTEROL IN HEALTH AND DISEASE AND ON THE BLOOD CHEMISTRY IN OSTEOMALACIA

BY

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In a series of observations (Hughes, Shrivastava and Sahai, 1929*a*) carried out during the cold weather of 1928-29 the serum calcium was found to be higher in healthy residents of the Punjab than in inhabitants of temperate regions. It was suggested that the cause of this was the great total amount of solar ultra-violet radiation to which the former were subjected. In 16 Indians the values ranged from 11·91 to 13·08 mg. per 100 c.c. with an average of 12·51. Corresponding figures for 8 Europeans resident in Lahore were 11·28, 12·4 and 11·74. Estimations were made by Kramer and Tisdall's method (1921) the precipitate of calcium oxalate being allowed to stand for 24 hours. The values recorded by workers in temperate climates using this method or some modification of it usually vary from 9 to 11 mg. per 100 c.c.

Since these results were published we have made further observations on the serum calcium in normal Punjabis and again subjected the method of estimation to various tests. We thought this desirable in view of the fact that other workers in the tropics had found lower values for serum calcium than we did. Kestner and Borchardt (1929) found that in Europeans in the Cameroons the values varied between 7·17 and 8·7 mg. per 100 c.c. and Wills and Mehta (1930) state that the average figure for 16 normal Indians in Bombay was 10·1 mg.

After satisfying ourselves as to the purity of the reagents and the accuracy of the standard solutions we checked the method in the following ways —

1 The amount of calcium in a solution of calcium chloride was estimated both by a macro-method and by that used in our experiments A comparison of the findings always showed agreement within narrow limits Thus a solution in which 660 mg were found by the former method yielded 656 mg by the latter

2 The calcium content of horses' serum and of a solution of calcium chloride was determined and amounts found by analysis in mixtures of these compared with the calculated figures The results never differed by more than 2 or 3 per cent, thus —

Horse's serum (Calcium in mg per 100 c c)	Calcium chloride solution (Calcium in mg per 100 c c)	Equal parts of serum and calcium chloride solution (Calcium in mg per 100 c c)	
		Experimental	Calculated
13 95	13·2	13 80	13·57

3 The question as to whether some magnesium is precipitated and estimated as calcium was investigated The possibility of this occurring has been pointed out by Hendriks (1929) We found that magnesium oxalate is not thrown out of solution if the serum is sufficiently diluted and excess of ammonium oxalate not used for precipitation Both these conditions are fulfilled in Kramer and Tisdall's method

4 Observations were made on the effect of varying the time during which the precipitate of calcium oxalate is allowed to stand We found that it made little or no difference whether the precipitate stood for ½ hour or for 24 hours and that in some instances the ½ hour precipitate was actually slightly the greater (Table I) —

Table I

No	Calcium in mg per 100 c c of serum (human)	
	Time allowed for precipitation 30 minutes	Time allowed for precipitation 24 hours
1	12 1	12·9
2	12 5	12·5
3	13 3	13 6
4	13·7	13·5
5	13 1	12·4
6	13·7	13 4

These results are in agreement with those of Tisdall (1923) On the other hand Warner (1930) states that at least 16 hours must be allowed for complete precipitation of the calcium as oxalate Loucks and Scott (1929) are also of opinion that complete precipitation does not occur in less than 16 hours owing to the difficulty of precipitating calcium from organic compounds It may be the case that in certain circumstances the precipitate is formed more readily than in others Warner has also drawn attention to the necessity of separating the serum from the blood soon after it is shed If this is not done the serum calcium value falls rapidly owing apparently to the calcium going into the clot He states that neglect of this precaution and failure to allow a sufficiently long time for precipitation of the calcium as oxalate 'account for many of the anomalous results of calcium estimations recorded in the literature' He obtained values ranging from 9 64 to 11 50 in six healthy medical students in London In all our estimations the serum was separated less than ½ hour after the blood was shed

Having checked the method of estimation we determined the concentration of serum calcium in seven normal Indians resident in Lahore, and obtained results which agreed with our previous findings The values ranged from 11 1 to 12 7 In two other apparently healthy individuals higher values were found but on investigation it was discovered that the subjects were not really normal It is interesting to note that Hess and Lewis (1928), working in New York in the spring and early summer on the effect of irradiated ergosterol on the serum calcium of normal infants, found high percentages in some cases and suggested that they were partly due to the effect of solar radiation

Effect of vitamins A and D on the serum calcium and inorganic phosphorus and on the plasma cholesterol

Further observations were carried out on these points, the methods used being those already described (Hughes, Shrivastava and Sahai, 1929*a*) In the case of hospital patients all blood samples were taken before the morning meal Blood was obtained from normals after breakfast Vitamin D was given in the form of Vigantol or Radiostol The source of vitamin A was Radiostoleum which also contains vitamin D We are indebted to the manufacturers for free supplies of these preparations

Previous experiments showed that vitamin D sometimes raised the serum calcium in patients whose initial percentages were within normal limits A rise in the blood cholesterol was also occasionally observed Of the observations now recorded two were made on normal subjects and in all the cholesterol content of the plasma, not of the whole blood, was determined The results are shown in Tables III and IV, and in general confirm the previous work No cases of osteomalacia are included An appreciable rise (over 10 per cent) in serum calcium was obtained with vitamin D alone in two individuals and with vitamins A and D in one Sometimes a fall resulted The inorganic phosphorus rose appreciably in 4 persons on vitamin D and in one on both vitamins, but in only one case was a high phosphorus content associated with

a rise in calcium The plasma cholesterol increased 10 per cent or more in three patients on irradiated ergosterol and in one on Radio-toleum A marked fall in cholesterol occurred in two patients receiving vitamin D In these the initial percentages were rather high In general the effect of vitamins A and D on these constituents of the blood did not seem to differ from that of vitamin D alone

One of the patients (No 6, Table III) whose plasma cholesterol was low (85 mg) was suffering from epilepsy A hypo-cholesterolæmia has been shown (Gosden, Fox and Brain, 1929) to be a feature of this condition and Gosden and Fox (1929) found that epileptics have often a low tolerance to lævulose It has therefore been suggested that the low cholesterol content of the blood in this disease is the result of liver deficiency In patient No 6 the tolerance to lævulose was markedly defective In view of the effect of the anti-rachitic vitamin in raising the blood cholesterol in some subjects, experiments were made to determine the influence of this vitamin on the glycogenic function in this individual After administration of Vigantol for 14 days the lævulose tolerance curve became normal although there was no change in the cholesterol, calcium or phosphorus Eight days later the fasting blood-sugar was high but the rise after 40 grammes of lævulose was small The plasma cholesterol had almost doubled and the serum calcium fallen from 16 5 to 12 0 These results are shown in Table II

TABLE II

Date	Calcium in mg per 100 c.c. serum	Inorganic phosphorus in mg. per 100 c.c. serum	Plasma cholesterol in mg. per 100 c.c.	Blood-sugar in mg. per 100 c.c. at ½ hourly intervals after ingestion of 10 gm. lævulose					
				Initial	½ hour	1 hour	1½ hours	2 hours	Rise in mg. per cent
21-10-29	10·0	4·4	85	0·115	0·173	0·189	0·200	0·179	55
4-11-29	16·5	4·2	85	0·111	0·131	0·131	0·127	0·113	17
12-11-29	12·0	4·4	154	0·170	0·182	0·181	0·178	0·182	12

Effect of vitamin D and of vitamins A and D on the blood chemistry in four cases of osteomalacia

Observations were made on the changes in serum calcium and inorganic phosphorus and on the plasma cholesterol in four cases of osteomalacia during administration of vitamin D plus, in one case, vitamin A The patients under the care of one of us (H) Table V shows the results

In two patients, Nos 1 and 2, there was marked hypocalcæmia on admission and the inorganic serum phosphorus was well above normal Both were tetany In the other two the calcium value was somewhat low but phorus was within normal limits No signs of tetany were present

TABLE III

Effects of vitamin D

Nos 1 to 7 received 'Radiostol' in daily doses of 2 c c and Nos 8 to 11 'Vigantol' in daily doses of 1 2 c c

No	Date	Name	Disease	Serum calcium in mg per 100 c c	Inorganic serum phosphorus in mg per 100 c c	Plasma cholesterol in mg per 100 c c
1	15-10-29	C R	Pulmonary tuberculosis	14 5	4 2	120
	21-10-29			16 0	4 4	188
	29-10-29			12·0	3·7	169
2	16-10-29	S R	Do	12 8	3·4	231
	24-10-29			12 5	4 7	162
3	18-10-29	A D	Do	14 5	4 4	169
	22-10-29			13 8	5 3	163
	30-10-29			16·0	5·4	149
4	23-10-29	S S	Do	13 5	4 6	152
	29-10-29			14 5	5·2	131
5	19-10-29	M H	Do	13 0	4 2	120
	25-10-29			12·5	3·6	189
6	21-10-29	B S	Epilepsy	16 0	4 4	85
	4-11-29			16 5	4 2	85
	11-11-29			12·0	4 4	154
7	24-10-29	N S	Nephritis	13 0	4 5	235
	1-11-29			12·3	4·6	150
	11-11-29			11 8	3·6	157
8	14-11-29	R S	Malaria	12 8	3 3	149
	2-12-29			11 3	5 0	146
9	20-11-29	J	Chronic malaria	16 4	4 1	106
				11 3	5 3	119
10	29-11-29	S S	Pulmonary tuberculosis	11 7	4 1	92
				10·2	4 4	108
11	2-12-29	G S	Do	12·3	4 1	85
	11-12-29			11 1	3 8	92

No 1 was at first given Radiostoleum and was later put on Vigantol The other 3 were given Vigantol only The diet contained an abundance of calcium and phosphorus

It is seen that in all four a rise in the serum calcium was produced The inorganic phosphorus was increased in all except No 1 in whom the changes were irregular The plasma cholesterol also underwent irregular fluctuations in this patient In Nos 2 and 3 the cholesterol fell and in No 4 it rose In the patients with low initial calcium values the increase in this element in the

[illegible] Improvement, however, as revealed both subjectively and by [illegible] progressive even when the serum calcium was stationary and below [illegible] This shows that the delayed rise was not due to non-absorption from the gut but rather to the fact that deposition in the bones almost kept pace with absorption Calcium enters the blood both from the intestine and from the bone depots and leaves it to be deposited in the skeleton or excreted The level of the blood calcium in osteomalacia is therefore not necessarily an indication of the severity or progress of the disease Thus a state of hypercalcæmia may be found in severe untreated cases (Hughes, Shrivastava and Sahai, 1929*b*, Case 10) owing presumably to excessive liberation of lime from the skeleton, such as occurs in hyper-parathyroidism, or it may occur during administration of vitamin D owing to increased absorption

TABLE IV

Effects of vitamins A and D

(Radiostoleum in daily doses of 2 c c)

No	Date	Name	Disease	Serum calcium in mg per 100 c c	Inorganic serum phosphorus in mg per 100 c c	Plasma cholesterol in mg per 100 c c
1	6-11-29	S S	Pulmonary tuberculosis	11·6	5·1	132
	18-11-29			11·7	5·2	112
	21-11-29			12·3	5·3	108
2	6-11-29	A D	Do	12·1	4·8	136
	19-11-29			11·1	5·1	123
3	7-11-29	B S	Do	14·2	3·2	54
	18-11-29			15·5	4·2	117
	21-11-29			10·5	4·4	113
4	13-11-29	B	Normal	12·7	3·9	113
	27-11-29			15·2	3·5	107
5	4-12-29	M	Do	12·7	3·9	109
	18-12-29			11·5	4·2	112

A study of the blood chemistry in treated and untreated cases would lead one to infer that in this disease there is interference with the normal exchange of calcium and phosphorus between the blood and the bones, as well as defective absorption of these elements from the intestine and that some factor other than deficiency of vitamin D plays a part in its causation In a previous paper (Hughes, Shrivastava and Sahai, 1929*b*) we gave reasons for assuming that avitaminosis A was partly responsible Harris (1930) holds that, while vitamin D influences calcification in cartilage, true osteogenesis is determined by vitamin A alone Improvement in Case 1 was most marked when vitamin A

TABLE V

No	Date	Patient	Age	Age at onset	Clinical notes	Treatment	Serum calcium in mg per 100 c.c. of serum	Inorganic phosphorus mgrms per 100 c.c. of serum	Plasma cholesterol in mg per 100 c.c.	Remarks
1	18-12-29	P. W.	16 years	10 years	Great deformity of the pelvis and bones of the legs. Tetany in hands and feet. Had undergone Cæsarean section. *X*-rays showed marked deficiency of lime salts in long bones and pelvis	Radiostoleum from 18-12-29	5·55	5·8	127	No treatment before 18-12-29
	3-1-30					Vigantol from 22-1-30	8·95	4·8	293	Tetany disappeared. Marked improvement. Bones denser
	18-1-30						8·70	5·7	125	Radiostoleum stopped and Vigantol started from 22-1-30
	5-2-30						9·55	5·2	180	
	18-2-30						9·6	4·09	144	Put on alkalis from 18-2-30. Urine alkaline on 19-2-30. Chvostek positive on 19-2-30. Complained of pains in the legs, etc., on 21-2-30. Alkalis stopped on 21-2-30. Chvostek almost gone on 22-2-30
	5-3-30						11·1	4·47	151	General improvement. New bone formation under periosteum

TABLE V—*concld*

No	Date	Patient	Age	Age at onset	Clinical notes	Treatment	Serum calcium in mg per 100 c c of serum	Inorganic phosphorus in mgms per 100 c c of serum	Plasma cholesterol in mg per 100 c c	REMARKS
2	25-3-30	S	15 years	5 years	Marked deformity of pelvis and bones of the legs Tetany in the hands only Deficiency of lime salts in pelvis and long bones on *X*-ray examination	Vigantol from 25-3-30	7 07	4 18	100	No treatment before 25-3-30
	29-3-30						8·08	5 1	116	Tetany gone Chvostek negative
	4-4-30						7 5	5 0	68	No tetany
	15-4-30						9 95	5 9	94	Bones somewhat denser
3	4-4-30	N	15 years	13 years	Pelvis and long bones deformed Amenorrhœa	Vigantol from 5-4-30	9 9	3 4	131	No treatment before 5-4-30
	17-4-30						11 4	3 99	62	Bones denser Menstruation started
	8-5-30						11 5	4 4	61	
4	17-4-30	D D	14 years	9 years	Pelvis and long bones deformed Enlargement of epiphyses at wrists Rickety rosary	Vigantol from 18-4-30	9·8	3 3	75	No treatment before 18-4-30
	8-5-30						11 6	4 4	170	Bones denser

was given along with vitamin D In some cases a probable causative agent is a high cereal diet which, as Mellanby (1930) has shown, aggravates the effects of a shortage of fat-soluble vitamins When there is an increased demand for calcium and phosphorus such as occurs during pregnancy and lactation exacerbations of the disease are to be expected A proper evaluation of all the factors concerned can only be obtained by determination of the calcium and phosphorus balance of patients under various conditions

In Case 1 all signs of tetany had disappeared when the serum calcium had reached 8 95 mg per 100 c c and in No 2 when it reached 8 1 mg When the calcium had risen to 9 6 mg in No 1, administration of large doses of alkalies for 24 hours enabled a positive Chvostek's sign to be elicited It is the amount of ionized calcium in the blood and not the amount of total calcium which is concerned with the production of tetany and the number of calcium ions is reduced in a condition of alkalosis Under ordinary circumstances about 1/5 of the serum calcium is in the ionic state In our previous series of cases calcium values of 9 2 and 9 77 were found in isolated observations on patients who were said to have had tetany In one of these spasmodic twitchings of the fingers were seen during the withdrawal of blood from the vein The ordinary tests for latent spasmophilia were unfortunately not done, but if this condition was present with a serum calcium of more than 9 it was probably due to alkalosis *

The inorganic phosphorus was above normal in the two low calcium cases and at the upper limit of normal in the other two In the high calcium case in the previous series it was low These facts suggest that a sort of reciprocal relationship may exist between the serum calcium and inorganic phosphorus in untreated patients Findings in other cases, however, show that this relationship is not universal

The cholesterol findings are still without an adequate explanation Various ideas suggest themselves such as the relationship of cholesterol to fat absorption, to calcium deposition or to the presence of fat-soluble vitamins in the liver but there is no evidence at present to support any of these

Conclusions

1 It has been confirmed that the serum calcium in normal residents of the Punjab is higher than in normal inhabitants of temperate climates

2 Oral administration of vitamin D or of vitamins A and D produced a rise in the serum calcium in 3 out of 16 individuals whose initial serum calcium was within or above normal limits The plasma cholesterol was appreciably increased in 4, in two of whom it was originally subnormal In two subjects a high plasma cholesterol fell when vitamin D was given

* Hunter (*Lancet*, 1930, I, p 999), records a positive Trousseau sign in a patient suffering from idiopathic steatorrhœa when the serum calcium was 10·8 mg per 100 c c

3 In a patient with idiopathic epilepsy in whom the tolerance to lævulose was defective, administration of vitamin D was followed by improvement in lævulose tolerance and by an increase in the plasma cholesterol from a subnormal to a normal level

4 Observations were made on the effect of vitamins A and D on four cases of osteomalacia The relationship of these vitamins to the pathogenesis osteomalacia is discussed

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THE INFLUENCE OF AGE AND TEMPERATURE OF STORAGE ON THE STRENGTH OF CHOLERA VACCINES

BY

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WHILE examining several samples of cholera vaccines manufactured in various laboratories in India it was strikingly noticed that the strength as judged by opacity was actually considerably lower than was stated on the label itself Such discrepancy was also found in the case of cholera vaccines prepared in German and American laboratories This meant apparently that either there has been an error in the standardization of the vaccine or there has been a deterioration in the strength of the vaccine since it was prepared and standardized Obviously the subject is one on which some precise information is desirable if the standardization of the vaccine is to be performed accurately

W W C Topley (1926) reviewing H C Brown's letter (1925) to the *Lancet* observes 'Most of us who have an extended experience of counting bacteria will probably agree that the opacity method, carried out with all possible precautions and in the hands of an experienced worker, is capable of giving results of a high degree of accuracy' While the above view may be taken as typical of the opinion of bacteriologists in this country also, the writer has reason to think that no uniformity exists in the time that may be permitted to elapse between the making of the cultural emulsion and the determination of the opacity test While preparing small quantities of autogenous vaccines for therapeutic purposes it is customary to carry out the opacity test as soon as the emulsion is made This, however, is rather difficult in large scale manufacture of prophylactic vaccines prepared from several strains In the latter case it is more convenient to defer the standardization till the contents of the culture bottles are tested for purity and sterility and pooled

together in stock vaccine bottles Castellani and Chalmers (1919) for instance allow the carbolized vaccine to stand at room temperature for 24 hours before standardizing The practice in some laboratories in India is to carry out the test on the fourth or fifth day when the stock vaccine bottles are passed for sterility This makes it necessary to determine if the vaccine has not undergone any alteration in its opacity during the period elapsing between the preparation of the emulsion and the carrying out of the test for its strength

Technique employed

Douglas agar medium (trypsinized mutton) and agar roll cultures were employed After eighteen hours' incubation the growth was emulsified in normal saline The opacity reading was at once taken and the emulsion was carbolized and left for varying periods at a room temperature of 90°F During the first week the opacity readings were taken daily and subsequently at weekly intervals for a month In some experiments the vaccine was kept at room temperature protected from light

Results of storage of cholera vaccine at room temperature

In all 29 experiments were carried out for ascertaining the variations in the opacity of the vaccine during storage at room temperature In only 4 experiments did the vaccine remain unaltered even for a day while kept at the room temperature In even these four cases a marked reduction in opacity took place after two days In all the remaining experiments a progressive reduction in opacity was noticed beginning from the first day and up to a week The reduction in opacity subsequently is not so rapid or progressive, the biggest drop having occurred in the first week only Of 29 vaccines examined 17 showed on preparation a strength of or above 40,000 millions per c c, but after a day's storage at room temperature only 2 showed a strength of even 40,000 millions and after a week's storage 28 samples showed a strength of 15,000 millions per c c or less The above figures markedly bring out the loss of strength as judged by opacity of the carbolized cholera vaccines when kept at room temperature in the plains The average strength of all the 29 vaccines at the time of preparation and after storage at the room temperature for a month was found to be as follows —

Freshly made			49,000 millions per c c			After	6	days	16,000 millions per c c	
After	1	day	29,000	„	„	„	14	„	15,000	„ „
„	2	days	25,000	„	„	„	21	„	13,000	„ „
„	3	„	19,000	„	„	„	28	„	12,000	„ „
„	4	„	18,000	„	„					

To find out how far the reduction in opacity could be arrested by maintaining at a low temperature, 18 samples were kept in cold storage at 32°F, a corresponding sample being kept at the room temperature (90°F) The results show a great improvement Even after a month's storage the reduction

is found to be less than what took place in a day at the room temperature The average strength for the 18 samples kept at 32°F was as follows —

Freshly made		49,000 millions per c c			After	6	days	37,000 millions per c c	
After	1 day	41,000	„	„	„	14	„	36,000	„ „
„	2 days	39,000	„	„	„	21	„	33,000	„ „
„	3 „	38,000	„	„	„	28	„	32,000	„ „
„	4 „	38,000	„	„					

While the low temperature does not altogether prevent the reduction in strength, it, however, considerably reduces the amount of the lysis or diminution in opacity

Another interesting point noticed in the course of the above experiments, is that in the case of the carbolized cholera vaccines the reduction in opacity during storage at room temperature is much greater in the case of vaccines of high strength than in the cases of vaccines of low strength Taking the 29 experiments the vaccines were divided into two lots, one showing a strength of 45,000 to 80,000 millions per c c (average 59,000 millions per c c) and another lot showing a strength from 30,000 to 40,000 millions per c c with an average strength of 35,000 millions per c c The reduction in opacity in each lot was worked out separately The following table shows that the percentage reduction is greater in the case of the stronger vaccine than in the weaker one —

TABLE I

Percentage reduction in opacity of carbolized cholera vaccine during storage at room temperature for varying periods after preparation

High strength	Low strength	High strength	Low strength
After 1 day 47 per cent	23 per cent	After 6 days 73 per cent	54 per cent
„ 2 days 56 „	34 „	„ 14 „ 74 „	60 „
„ 3 „ 68 „	46 „	„ 21 „ 78 „	63 „
„ 4 „ 69 „	48 „	„ 28 „ 80 „	66 „

In either case, as already mentioned, the biggest drop occurs in the first twenty-four hours After 48 hours also a fairly big drop takes place Subsequent to that only a slight but progressive reduction in opacity is noticed for a month

It was thought that the above autolysis may be due either partly or totally to the influence of light Several experiments were conducted to determine the influence of light on the opacity of the vaccine up to a period of six months It was found that in the causation of lysis the influence of light was negligible Whether exposed to light or carefully protected from it, the vaccines kept at room temperature underwent considerable reduction in strength Even at the low temperature of 32°F, exposure to light had no effect in influencing even

TABLE II

Strength of cholera vaccine immediately after preparation and after storage for varying periods at room temperature 90°F and at 32°F

Days	Room temperature									Cold storage								
	0	1	2	3	4	6	14	21	28	0	1	2	3	4	6	11	21	28
Exp 1	50	25	20	15	15	10	10	10	10									
„ 2	40	20	15	10	10	10	10	10	10									
„ 3	45	25	20	15	15	15	10	10	10									
„ 4	50	30	20	15	10	10	10	10	10									
„ 5	60	30	20	15	15	10	10	10	10									
„ 6	50	20	20	15	12 5	10	10	10	10									
„ 7	50	20	20	15	12 5	12 5	10	10	10									
„ 8	60	30	25	20	12 5	12 5	10	10	10									
„ 9	45	20	20	10	10	10	10	10	10									
„ 10	40	20	20	10	10	10	10	10	10									
„ 11	50	20	15	10	10	10	10	10	10									
„ 12	40	30	20	20	20	10	10	10	10	40	40	35	40	40	40	35	30	30
„ 13	30	30	25	25	25	20	20	20	15	35	35	40	35	35	30	30	30	25
„ 14	50	50	40	30	30	20	20	20	15	40	40	40	40	40	40	35	35	35

15	30	30	25	20	20	20	15	15	30	30	30	30	30	30	25	25	25	25	25	25	25	25	25	25	25
16	30	30	20	20	20	15	15	15	30	30	30	30	30	30	30	30	30	25	25	25	25	25	25	25	25
17	40	30	30	30	30	20	20	15	40	40	40	40	10	40	40	40	40	40	40	30	30	30	30	30	30
18	30	25	25	15	15	15	15	15	40	35	35	35	30	30	30	30	30	30	30	30	30	30	30	30	30
19	40	35	25	20	20	20	15	10	40	35	35	35	35	35	35	35	30	30	30	30	30	30	30	30	25
20	50	30	30	20	20	20	15	15	60	55	55	55	50	50	50	40	40	40	40	40	40	40	40	40	35
21	40	30	30	20	20	20	10	10	40	35	35	35	35	30	30	30	30	30	30	30	30	30	30	30	25
22	30	25	20	20	20	15	15	10	30	25	25	25	20	20	20	20	20	20	20	20	20	20	20	20	20
23	30	20	20	20	10	10	10	10	30	25	25	25	20	20	20	20	20	20	20	20	20	20	20	20	20
24	80	40	30	30	30	20	20	15	80	60	60	60	60	60	60	60	60	60	60	60	60	60	60	55	55
25	80	50	40	40	40	30	30	25	70	50	50	50	50	50	50	50	50	50	50	50	50	50	50	45	45
26	70	40	30	20	20	20	20	15	70	50	50	50	50	50	50	50	50	50	50	50	50	50	50	40	30
27	70	30	30	20	20	20	20	15	70	50	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
28	70	30	30	20	20	20	20	15	65	55	45	45	45	45	45	45	45	45	45	45	45	45	45	40	40
29	70	40	30	20	20	20	20	15	70	50	40	40	40	40	40	40	40	40	40	40	40	40	40	35	35

Note—The results are expressed in 1,000 millions, e g, 50 = 50,000 millions

the comparatively slower autolysis of the vaccine It was, however, found that exposure to light rapidly turned the vaccines brownish The original colour of the vaccine was maintained for a much larger period when the vaccine was completely protected from light during storage either at the room temperature or in the refrigerator

Conclusion

(1) Carbolized cholera vaccines rapidly undergo progressive autolysis and reduction in opacity when kept at room temperature in the plums Even within twenty-four hours the opacity may be considerably reduced, especially if the vaccine is of high strength In about a week's time the strength may be reduced to half the original strength and after a month may be only one-fourth or one-fifth the original strength, as adjudged by opacity This autolysis may be greatly retarded by keeping the vaccine in a refrigerator at 32°F To obtain accurate results it is essential to standardize the vaccine immediately after its preparation

(2) Exposure to light does not seem to be the cause of the autolysis as even in the dark similar autolysis was observed when the vaccine is kept at the room temperature Exposure to light, however, has the effect of altering the colour of the vaccine to dark brown

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SOME FINDINGS AND OBSERVATIONS IN AN ANOPHELINE MALARIA INFECTIVITY SURVEY CARRIED OUT IN THE CACHAR DISTRICT OF ASSAM

BY

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INTRODUCTION

COVELL (1927) in the introduction to his Memoir 'A critical review of the data recorded regarding the transmission of malaria by the different species of Anopheles, with notes on distribution, habits and breeding-places' stated that in India since 1902 comparatively little research in this direction has been carried out with the exception of the work of Bentley in Bombay, and of Gill in the Punjab During the early stages of my investigations in 1926, on Assam's malaria problems, it became apparent to me, on reviewing the available literature at my disposal, that comparatively little research had been carried out on the relative infectivity of the Anopheles of Assam Realizing the importance of more complete data, if Assam's malaria problems were to be tackled in an efficient manner, from point of view of species control, I decided to undertake an extended infectivity survey

The findings and observations, which are recorded in this publication, are based mainly on dissections of adult Anopheline mosquitoes, caught in Nature, from April 1st, 1927 to March 31st, 1930

One month before I began this survey, one of my establishment proceeded to the School of Tropical Medicine, Calcutta, for training in mosquito dissection and with the object of being shown Culicine mosquitoes infected, experimentally, with bird malaria Unfortunately, at that period, infected mosquitoes were not available for demonstration purposes As neither myself nor any of my staff had seen either oocysts or sporozoites in their unstained natural state in dissected mosquitoes, the initial stages of this investigation were indeed very trying and have made me sincerely appreciate the researches and discovery of Sir Ronald Ross In the early stages of this work, before I became thoroughly acquainted with the normal and abnormal contents and appearances of dissected

mosquitoes, I encountered the usual pitfalls awaiting inexperience in a specialized subject In these early days, I imagined I had seen oocyst infection in *A vagus* and in *A kawari*, but enlightenment came with experience and soon enabled me to recognize genuine malarial infection from pseudomorphous objects During the three years' survey twenty-five members of my anti-malaria establishment were at one period or another engaged in mosquito dissection The members of my anti-malaria establishment, who were engaged in this infectivity survey, have all been recruited in this district and mainly from hospital establishments in the Labac Medical Practice Four dissecting microscopes were available in my laboratory until the end of December 1927, thereafter the survey was carried on with two dissecting microscopes The total number of adult Anopheline mosquitoes caught in Nature, and dissected, in the period under review was 42,300 and comprised 18 species The mosquitoes were caught practically entirely in collecting tubes in human habitations, cowsheds and hospitals In the early stages of this investigation I experimented with various types of mosquito traps, but the traps employed were cumbersome to handle and unsatisfactory as collecting media I soon discarded traps, as I found it was more practical to collect the number of Anopheline mosquitoes required for dissection daily in collecting tubes The collecting tubes were sent early every morning from various tea estates in the Cachar district to the Labac Laboratory, and the mosquitoes were kept in globes, covered with gauze, until they had digested their blood meal In order to stimulate interest amongst the local labourers employed on tea estates, but mainly to facilitate collecting mosquitoes in human habitations, I gave, during 1927, a reward of one rupee to each owner of a house or cowshed, in which an infected Anopheline mosquito had been captured

Thereafter, owing to the scarcity of adult *A minimus* and *A ramsayi* on gardens, where anti-larval measures had been instituted, I increased the reward to two annas for each adult *A minimus* and *A ramsayi* caught, in addition to one rupee for any Anopheline mosquito found infected with malaria The mosquitoes collected and dissected were, however, principally from areas where anti-larval measures had not been applied In 1927 the number of tea estates, which were being controlled by anti-larval measures, was very limited, the number was greatly increased in 1928, and again more estates came under control during 1929 In the controlled tea estates it was extremely difficult to find adult *A minimus* mosquitoes for dissection purposes The scarcity of *A ramsayi*, in the dissection records after 1927, was due to the eradication of the main breeding areas of this species early in 1928

Some findings and observations

The number of each species dissected and the malaria findings are recorded in Table I

The results of dissections and the numbers of the various species dissected, during the various months of the year, are recorded in Table II to Table XIII

TABLE I

Anophelme mosquitoes dissected from 1st April, 1927 to 31st March, 1930

No	Name of species	Number dissected	Results	
			Glands infected	Gut infected
1	*A minimus*	3,874	27	59
2	*A ramsayi*	287	1	
3	*A hodi*	2,094		2
4	*A vagus*	7,601		
5	*A karwari*	9,242		
6	*A maculatus*	3,374		
7	*A jeyporiensis*	888		
8	*A culicifacies*	120		
9	*A tessellatus*	95		
10	*A aconitus*	1,661		
11	*A fuliginosus*	335		
12	*A philippinensis*	6,895		
13	*A hyrcanus*	5,461		
14	*A jamesii*	162		
15	*A barbirostris*	26		
16	*A aitkenii*	6		
17	*A leucosphyrus*	175		
18	*A theobaldi*	4		
	TOTAL	42,300	28	61

while on the graphs are recorded the maximum and minimum temperatures, the vapour pressure or absolute humidity, the rainfall, and the dates when mosquitoes were found in Nature to be infected with malaria In studying these findings, it will be seen that, in 42,300 dissections, only eighty-nine mosquitoes were found to be infected with malaria The infected mosquitoes were caught almost entirely in highly malarious sites Of the eighty-nine mosquitoes eighty-six belonged to the species *A minimus*, of which species 3,874 were dissected While, of the three remaining infected mosquitoes, one was *Anopheles ramsayi*, of which species 287 were dissected,

[illegible] were *Anopheles kochi* of which species 2,091 were dissected these eighty-nine infected mosquitoes, eighty-six were caught in human [illegible] while only three were caught in cowsheds The three infected [illegible] caught in cowsheds were two *A minimus* and one *A ramsayi* *A minimus*, with sporozoite infection in the salivary glands, also had oocyst infection of the gut but the latter are not included in Table I, under the heading 'gut' infection The maximum number of oocysts seen in an infected gut was 198 From the dissection records it would appear that *A minimus* is

Table II

Anopheline mosquitoes dissected during the month of January 1928, 1929 and 1930

No	Name of species	1928	1929	Total	Results	
					Glands infected	Gut infected
1	*A minimus*	100	106	38		
2	*A jeyporiensis*	17	28	18	1	
3	*A aconitus*	70	186	17		
4	*A vagus*	30	68	210	3	
5	*A kochi*	13	88	25	1	
6	*A hyrcanus*	31	171	66	2	
7	*A philippinensis*	28	98	156		
8	*A karwari*	61	365	194		
9	*A maculatus*		38	9		
10	*A jamesi*		1	2	17	
11	*A aitkeni*		1		3	
					1	
	TOTAL	383	1,150			
				2,628		

practically entirely responsible for t[illegible] transmission of malaria in the Cachar district of Assam The period, du[illegible] which *A minimus* was found to be infected with malaria in Nature, [illegible] from April 14th to December 22nd During this period 3,153 *A minim*[illegible] were dissected, the infection rate being 2 7 per cent, but actually the per[illegible] during which *A minimus* was found capable of transmitting malaria, t[illegible] when infected with sporozoites in the salivary glands was from May [illegible] to December 9th It would appear

therefore that there is, in Cachar, a definite period during the year, when mosquitoes do not become infected with malaria This finding is in keeping with the effects of climate on the life history of *A minimus* The effects of climate, on plant life in Assam, has been well described by Harler in the Quarterly Journal of the Scientific Department, Indian Tea Association As Harler's publication is equally of great importance, when applied to Assam malariology,

TABLE III

Anopheline mosquitoes dissected during the month of February 1928, 1929 *and* 1930

No	Name of species	1928	1929	1930	Total	Results	
						Glands infected	Gut infected
1	*A minimus*	64	41	34	139		
2	*A ramsayi*	1	15		16		
3	*A aconitus*	57	259	47	363		
4	*A jeyporiensis*	104	26	16	146		
5	*A kochi*	52	80	42	174		
6	*A karwari*	269	280	666	1,215		
7	*A hyrcanus*	112	183	137	432		
8	*A philippinensis*	72	179	274	525		
9	*A fuliginosus*	4			4		
10	*A vagus*	29	9	112	150		
11	*A barbirostris*	2	1		3		
12	*A maculatus*	4	38	22	64		
13	*A jamesi*		4	2	6		
14	*A leucosphyrus*			1	1		
	TOTAL	770	1,115	1,353	3,238		

especially from the point of view of rainfall and temperature, I take the liberty to quote his article freely In 1922 Harler wrote 'the monsoon in Assam differs considerably from that experienced generally in Northern India In provinces other than Assam, the drought as a rule continues till about the middle of June, and the rains cease towards the end of September The rain corresponds with the moving north of the thermal equator, and the steady ir

from the Central Asiatic plateau enormously accentuates the inflow of rain-bearing winds to North India

'In most parts of North India three definite seasons, cold, hot and wet, are distinguished, but in Assam there are roughly two, the cold, dry season and the wet warm one During the period March, April, May, the weather is

TABLE IV

Anopheline mosquitoes dissected during the month of March 1928, 1929 *and* 1930

No	Name of species	1928	1929	1930	Total	Results	
						Glands infected	Gut infected
1	*A minimus*	26	19	19	121		
2	*A ramsayi*		11		11		
3	*A jeyporiensis*	58	16	24	98		
4	*A aconitus*	62	101	15	178		
5	*A maculatus*	15	111	51	207		
6	*A kochi*	108	90	18	216		
7	*A karwari*	331	261	618	1,210		
8	*A hyrcanus*	153	156	103	412		
9	*A philippinensis*	50	80	203	333		
10	*A fuliginosus*	23	5	60	88		
11	*A vagus*	54	49	200	303		
12	*A barbirostris*	2	2	2	6		
13	*A tessellatus*	3	5		8		
14	*A jamesii*		2	2	4		
15	*A leucosphyrus*		1		1		
16	*A theobaldi*			2	2		
	TOTAL	915	939	1,407	3,261		

changeable, and is usually alternately hot and cool with a tendency to get hotter

'In June the uptake of air from the Tibetan plateau becomes steady, and the monsoon advances, so that up the Assam Valley a steady south-west wind

blows, and the sky is cloudy and overcast for two months In August and September there is a period of comparative equilibrium, and clear and cloudy skies alternate Early in August it is frequently observed that the breeze blows direct from the north October sees the monsoon definitely in retreat, and the wind blows steadily from the north So far as rainfall is concerned, there are two critical periods in the season The first is in March, April and

TABLE V

Anopheline mosquitoes dissected during the month of April 1927, 1928 *and* 1929

No	Name of species	1927	1928	1929	Total	Results	
						Glands infected	Gut infected
1	*A minimus*	8	104	121	233		1
2	*A kochi*	93	118	78	289		
3	*A vagus*	29	300	134	463		
4	*A karwari*	30	190	140	360		
5	*A maculatus*	4	144	410	558		
6	*A jeyporiensis*	1	61	13	75		
7	*A tessellatus*	1	19	15	35		
8	*A aconitus*	11	9	21	41		
9	*A fuliginosus*	7	28	6	41		
10	*A philippinensis*	21	24	47	92		
11	*A hyrcanus*	94	56	125	275		
12	*A leucosphyrus*	1	5		6		
13	*A barbirostris*		1	1	2		
14	*A culicifacies*		14	4	18		
15	*A jamesii*		1	4	5		
16	*A theobaldi*		1		1		
17	*A ramsayi*			1	1		
	TOTAL	300	1,075	1,120	2,495		1

May, and the other in September and October The rain seldom fails in the months between, but is usually in great excess

'*Temperature and humidity*'—Observations in connection with many plants show that the dominating factor in plant growth is temperature Below 42°F

plant growth ceases Closely correlated with temperature and with rainfall is the humidity of the atmosphere It is further necessary to distinguish between relative and absolute humidity In the cold weather, when a mist is lying about, the atmosphere is saturated, and the relative humidity is 100 If the temperature were suddenly raised about 10°F , the mist would clear and the relative humidity would fall, as it often does, to about 70, although there is still the same amount of moisture in the atmosphere, as there was, when the air was cooler, i e., the absolute humidity is unchanged

TABLE VI

Anopheline mosquitoes dissected during the month of May 1927, 1928 *and* 1929

No	Name of species	1927	1928	1929	Total	Results	
						Glands infected	Gut infected
1	*A minimus*	52	211	87	350	3	3
2	*A kochi*	91	10	39	190		
3	*A vagus*	53	350	98	501		
4	*A karwari*	106	44	59	209		
5	*A maculatus*	14	98	918	1,060		
6	*A jeyporiensis*	10	34	29	73		
7	*A culicifacies*	1	81	3	85		
8	*A tessellatus*	3	14	8	25		
9	*A aconitus*	24	10	11	15		
10	*A fuliginosus*	12	65	7	84		
11	*A philippinensis*	63	22	42	127		
12	*A hyrcanus*	251	51	27	329		
13	*A jamesii*	1		45	46		
14	*A leucosphyrus*		4	4	8		
	TOTAL	681	1,024	1,427	3,132	3	3

'During the nights in the rains the atmosphere is generally saturated, and the relative humidity is then 100, but the actual amount of moisture in the air is often three times the amount present in the cold weather, when the atmosphere is saturated This difference is not measured by the relative humidity which is merely the amount of moisture in the air compared with

that necessary to saturate the air at the same temperature This latter quantity varies with temperature, so that the same amount of moisture may correspond to any number of relative humidities The relative humidity varies greatly throughout the day, and may alter from 100 at 10 A M to 50 at noon, when the mist clears This is especially the case in the cold weather The absolute humidity seldom shows such variation, although it shows a steady

TABLE VII

Anopheline mosquitoes dissected during the month of June 1927, 1928 *and* 1929

No	Name of species	1927	1928	1929	Total	Results	
						Glands infected	Gut infected
1	*A minimus*	235	132	50	417	7	10
2	*A ramsayi*	27			27	1	
3	*A kochi*	19	63	49	131		
4	*A vagus*	23	224	230	477		
5	*A karwari*	142	100	294	536		
6	*A maculatus*	20	92	491	603		
7	*A jeyporiensis*	8	31	48	87		
8	*A aconitus*	8	3	12	23		
9	*A fuliginosus*	10	52	5	67		
10	*A philippinensis*	115	78	144	337		
11	*A hyrcanus*	112	86	94	292		
12	*A barbirostris*	1	4	1	6		
13	*A culicifacies*		16	1	17		
14	*A leucosphyrus*		27	15	42		
15	*A jamesi*		1	33	34		
16	*A tessellatus*		11	1	12		
	TOTAL	720	920	1,468	3,108	8	10

change over periods of days It will be understood that, before the absolute humidity of the air can be appreciably increased, there must be a hot period extending over several days, and, at the same time, the nights must not be cold enough to precipitate as dew all the extra moisture taken up It usually happens that right to the end of May, although the daily temperature rises to

90°F, the absolute humidity does not greatly increase, because the night temperatures fall to about 70°F In the monsoon no such fall occurs at night The question of humidity (humidity of air irrespective of rainfall) has an important bearing of the growth of tea as well as an insect and fungus attack'

My observations in Cachar have certainly shown me the importance of the effects of rainfall and of temperature on the bionomics of Anopheline

TABLE VIII

Anopheline mosquitoes dissected during the month of July 1927, 1928 *and* 1929

No	Name of species	1927	1928	1929	Total	Results	
						Glands infected	Gut infected
1	*A minimus*	266	218	32	516	2	7
2	*A ramsayi*	35			35		
3	*A kochi*	14	57	6	77		2
4	*A vagus*	31	54	136	521		
5	*A karwari*	125	197	276	598		
6	*A maculatus*	16	182	176	374		
7	*A jeyporiensis*	15	30	29	74		
8	*A aconitus*	1	1	2	7		
9	*A fuliginosus*	8	15	3	26		
10	*A philippinensis*	161	80	399	640		
11	*A hyrcanus*	88	19	127	234		
12	*A jamesii*	1	2	24	27		
13	*A aitkenii*	1	2		3		
14	*A leucosphyrus*	13	45	6	64		
15	*A tessellatus*		7	1	8		
16	*A theobaldi*		1		1		
	TOTAL	775	913	1,517	3,205	2	9

'Dur[illegible]nd especially on the bionomics of *A minimus* As in plant life, [illegible]he relative is a dominating influence on Assam Anopheline mosquitoes In [illegible] is often thre[illegible] cold dry weather, when the night temperatures drop below [illegible]sphere is sa[illegible]mosquitoes, and especially *A minimus*, are seldom seen in [illegible]y which is the adult stage During this period of the year I found

great difficulty in collecting sufficient Anopheline mosquitoes for dissection daily, whereas during the warm steamy weather there was a superabundance The increase in numbers begins with the rising night temperatures in March, reaches its peak point during the steamy monsoon season, and suddenly decreases with the falling night temperatures in November The mosquitoes (including *A minimus*) collected and dissected during the cold dry weather were found chiefly in cowsheds Cachar Anopheline mosquitoes, however, spend the greater

TABLE IX

Anopheline mosquitoes dissected during the month of August 1927, 1928 *and* 1929

No	Name of species	1927	1928	1929	Total	Results	
						Glands infected	Gut infected
1	*A minimus*	120	65	27	212	2	8
2	*A ramsayi*	19			19		
3	*A kochi*	35	53	52	140		
4	*A vagus*	33	105	559	697		
5	*A karwari*	268	270	314	852		
6	*A maculatus*	15	58	30	103		
7	*A jeyporiensis*	23	30	10	63		
8	*A aconitus*	2	6	1	9		
9	*A fuliginosus*	5	3	5	13		
10	*A philippinensis*	327	166	426	919		
11	*A hyrcanus*	186	131	103	420		
12	*A leucosphyrus*	1	14	5	20		
13	*A barbirostris*		3		3		
14	*A jamesi*		1	13	14		
	TOTAL	1,034	905	1,545	3,484	2	8

part of their life history during December, January and February in the larval stage Under laboratory conditions during December, January and February *A minimus* in the adult stage can survive for at least six weeks, whereas during the steamy monsoon season it is fortunate to survive for three weeks Again, under laboratory conditions I have found in the warm steamy weather that *A minimus* will digest its blood meal and feed on human blood daily, while

during the cold dry weather it is with difficulty that it can be induced to feed every three or four days These feeding experiments refer, of course, to mosquitoes, which have been hatched out in the laboratory During the past year I had no difficulty in infecting two out of three *A minimus* mosquitoes, fed on a subtertian gametocyte carrier, during the month of July 1929, but I have

TABLE X

Anopheline mosquitoes dissected during the month of September 1927, 1928 *and* 1929

No	Name of species	1927	1928	1929	Total	Results	
						Glands infected	Gut infected
1	*A minimus*	71	36	2	109	1	3
2	*A ramsayi*	68			68		
3	*A kochi*	24	15	25	64		
4	*A vagus*	126	209	514	849		
5	*A karwari*	220	157	216	593		
6	*A maculatus*	12	15	6	33		
7	*A jeyporiensis*	14	16	5	35		
8	*A aconitus*	1	2	3	6		
9	*A philippinensis*	327	277	117	1,021		
10	*A hyrcanus*	200	168	303	671		
11	*A aitkeni*	2			2		
12	*A leucosphyrus*	5	3	3	11		
13	*A barbirostris*		1		1		
14	*A fuliginosus*		1		1		
15	*A jamesi*			4	4		
	TOTAL	1,070	900	1,498	3,468	1	3

failed to infect a single mosquito in twenty of this species fed, during the daytime, on benign tertian, and on quartan, gametocyte carriers, when the night temperatures had dropped below 60°F during December, January and February (1929-1930) Again, from my dissection records, I find that 721 *A minimus* were caught and dissected between December 22nd and April 14th in what, from our present records, would appear to be the Cachar non-transmission

season, but, actually in Nature, malaria is apparently being transmitted when the night temperatures rise above 60°F during the months of March and April

In studying the graphs it will be seen that infected mosquitoes are found in Nature, after the night temperatures drop below 60°F The most obvious explanation is that these mosquitoes were infected before the night temperatures fell low enough to inhibit the feeding stimulus of mosquitoes in human habitations

TABLE XI

Anopheline mosquitoes dissected during the month of October 1927, 1928 *and* 1929

No	Name of species	1927	1928	1929	Total	Results	
						Glands infected	Gut infected
1	*A minimus*	219	12	100	331	1	6
2	*A ramsayi*	65			65		
3	*A kochi*	66	21	17	104		
4	*A vagus*	412	203	414	1,029		
5	*A karwari*	230	242	175	647		
6	*A maculatus*	4	13	8	25		
7	*A jeyporiensis*	16	6	15	37		
8	*A tessellatus*	1			1		
9	*A aconitus*	3	3	3	9		
10	*A philippinensis*	488	131	238	857		
11	*A hyrcanus*	430	274	571	1,275		
12	*A jamesii*	2		2	4		
13	*A barbirostris*	1		2	3		
14	*A leucosphyrus*	15	1	3	19		
	TOTAL	1,952	906	1,548	4,406	1	6

The effect of humidity on the infectivity of mosquitoes has been studied by several investigators Recently Mayne writes, 'Wenyon (1926) considers that there is no evidence that the factor of the effect of humidity of the atmosphere plays any part on the active development of plasmodia in mosquitoes Provided there is sufficient moisture in the air to enable the mosquito to live, the malaria parasite will develop normally He regards

temperature as a much more important factor than humidity, and refers to Gill (1921) who, however, has pointed out that the spread of malaria may be affected by the lack of humidity, because the mosquitoes, which ingest parasites, may not live long enough for sporozoites to appear in the salivary glands' Gill (1921) and Mayne (1928) have correlated the results of their infectivity investigations with the relative humidity of the atmosphere In the graphs submitted it will be observed that the vapour pressure or absolute humidity

TABLE XII

Anopheline mosquitoes dissected during the month of November 1927, 1928 *and* 1929

No	Name of species	1927	1928	1929	Total	Results	
						Glands infected	Gut infected
1	*A minimus*	382	58	207	647	10	13
2	*A ramsayi*	29			29		
3	*A kochi*	131	111	12	287		
4	*A vagus*	491	136	566	1,193		
5	*A karwari*	352	249	351	952		
6	*A maculatus*	21	4	23	48		
7	*A jeyporiensis*	22	16	6	11		
8	*A tessellatus*	1	1		2		
9	*A aconitus*	63	165	29	257		
10	*A fuliginosus*	8			8		
11	*A philippinensis*	603	61	175	839		
12	*A hyrcanus*	257	120	109	486		
13	*A jamesii*	3	1	1	5		
14	*A leucosphyrus*	1		2	3		
	TOTAL	2,364	925	1,511	4,800	10	13

shows a high degree of correlation with the maximum temperature during the rainy season and generally with the minimum temperature in the cold weather The question arises here, was it the fall in the absolute humidity, or was it the low average mean temperature, or again was it possibly unripe gametocytes, which inhibited the development of malaria infection in twenty *A minimus* mosquitoes, which were fed during the day-time on gametocyte

carriers in my laboratory, during the period of the year when the night temperatures had fallen below 60°F ? Hailer has pointed out, that, during the Assam cold weather, the relative humidity is high, and therefore relative humidity did not appear to be the factor in inhibiting the malarial cycle from developing in these mosquitoes These mosquitoes were fed during the day-time,

TABLE XIII

Anopheline mosquitoes dissected during the month of December 1927, 1928 *and* 1929

No	Name of species	1927	1928	1929	Total	Results	
						Glands infected	Gut infected
1	*A minimus*	329	104	119	552	1	8
2	*A ramsayi*	16			16		
3	*A lochi*	95	134	37	266		
4	*A vagus*	324	72	684	1,080		
5	*A karwari*	594	190	333	1,117		
6	*A maculatus*	138	58	56	252		
7	*A jeyporiensis*	29	24	10	63		
8	*A tessellatus*	1	3		4		
9	*A aconitus*	48	318	54	420		
10	*A fuliginosus*	2	1		3		
11	*A philippinensis*	714	64	145	923		
12	*A hyrcanus*	213	94	60	367		
13	*A jamesi*	1	2	7	10		
14	*A barbirostris*			2	2		
	TOTAL	2,504	1,064	1,507	5,075	1	8

as they could not be induced to feed at night-time, that is, temperature appears to be the main factor in controlling the feeding stimulus

In Assam, the accurate demarcation of the period of the year, when mosquitoes can become infected and transmit malaria in Nature, is of great importance, especially to employers of labour, who have to import non-immune labour to uncontrolled hyperendemic sites for so-called cold weather repair

work An example of this is brought out in a letter from an employer of labour, who recently wrote to me as follows, 'we have had two lots of freshly imported labour, one a batch of 75 men, who arrived on March 5th, and another a batch of 8 riveters, who arrived on March 26th The night temperature rose to over 60°F on March 14th, and has remained up since On the 4th of April the 75 men batch bolted *en bloc*, as a large number of them developed fever On the 18th April all the eight riveters were laid out with fever, and had to be sent back on the 22nd as absolute wrecks'

Although, so far, I have been unable to capture any infected mosquitoes during the month of March, practical experience has shown me that malaria is undoubtedly being transmitted on hyperendemic sites during this period of the year The essential factors are a high gametocyte index, close proximity to breeding-places of *A minimus*, and night temperatures over 60°F The difficulty in finding infected *A minimus* during March, April and May is also increased by the effects of rainfall, as it is during this period of the year that *A minimus* migrates from its winter to its summer resorts Further, the low degree of infectivity of *A minimus* in my dissection records during March and April was, to a large extent, due to this species being caught in areas with a low gametocyte index

When studying the graphs of total sick rates and malaria rates on malarious tea estates, it will be observed that these rapidly fall, when the night temperatures drop below 60°F But, again, an intimate knowledge of the spleen rates, parasite rates, and the health of tea garden labour forces, also of the breeding-places of *A minimus*, shows that much malaria is being transmitted during the months of October and November, and that many of these infections lie latent until the following March and April The explanation would appear to be that the resistance of the individual is increased during the cold dry weather This latency of malarial infection has already been demonstrated experimentally by Swellengrebel in Holland

Perhaps some of the most important points, which have emerged from this research, are our negative findings, especially with species such as *A maculatus* and *A aconitus*, which are regarded as dangerous natural carriers of malaria in the Federated Malay States

In Cachar, *A maculatus* is only prevalent on tea estates situated near the foot-hills I have made a special effort, especially during 1929, to investigate the degree of infectivity of this species on a tea estate where the spleen rate, amongst garden born children, was, in 1928, over 80 per cent The spleen rate on this estate has been checked independently by Sir Malcolm Watson, Dr Bentley, Dr Bruce Mayne and by Dr Meek, Medical Officer in charge of the garden The following are the comparative findings in dissections of *A minimus* and *A maculatus*, which were caught, on this estate, during the period of the year, in Cachar, when mosquitoes have been found to be infected with malaria in Nature, that is from April 14th to December 22nd

Year	Name of species	Number dissected	Results	
			Glands infected	Gut infected
1927	*A minimus*	231	5	8
	A maculatus	94	Nil	Nil
1928	*A minimus*	142	4	2
	A maculatus	158	Nil	Nil
1929	*A minimus*	13	Nil	Nil
	A maculatus	1818	Nil	Nil

It will be seen that 19 *A minimus* were found to be infected in 386 dissected, whereas 2,070 *A maculatus*, caught in the same area, were found to be uninfected It became early apparent, when searching for *A maculatus* on this estate, that this species was extremely difficult to find in human habitations, while it could be readily caught in cowsheds This, of course, has been my experience with our common Cachar species such as *A aconitus*, *A philippinensis*, *A vagus*, *A hyrcanus*, *A karwari* and *A kochi* *A minimus*, however, except during the cold weather, is more easily found in human habitations, than in cowsheds It is most easily caught, after having fed, on the walls and sometimes on the ceiling, in the sleeping room of two-roomed coolie huts from 8 P M until daybreak The smoke from cooking fires in the early part of the evening in a one-roomed hut is apparently the factor in limiting the numbers caught in this type of habitation That *A minimus* is a human blood lover is obvious, when feeding experiments are carried out in a laboratory During the warm monsoon weather I find there is no difficulty in inducing *A minimus* to feed on human blood, but all our other Cachar species, with the possible exception of *A culicifacies*, which is an uncommon species in this district, are, in comparison, more or less hunger-strikers, and die early in captivity Although *A minimus* is a human blood lover, I find that it can be induced to feed on cow's blood in a laboratory In Nature, *A minimus* must often be forced to feed on blood other than human On several occasions I have visited at night-time, with members of my staff, known breeding areas of *A minimus* and other Anopheline species, which were over a mile away from human habitations On these occasions *A minimus*, *A maculatus*, *A aconitus*, *A philippinensis*, *A hyrcanus*, *A karwari* and *A kochi*, also many Culicines were caught regaling themselves with blood meals on myself or on my staff, but it was obvious that, in the absence of human blood, *A minimus* in these remote breeding areas could find available animal blood, as jackals and foxes were seen quite close to these *A minimus* breeding-places

The part played by cattle and other animals in attracting certain species of Anopheline mosquitoes, known to be carriers of malaria in Nature, and

thereby limiting their attacks on human beings, is indeed a fortunate happening in this district

In the Federated Malay States, I find, on investigation, that the labour forces employed on the Rubber Estates are engaged on short term contracts, and do not keep large herds of cattle, as the settled tea garden labour forces do in Assam, hence *A maculatus* and *A aconitus*, in the comparative absence of cattle, apparently turn their attention to mankind, and thereby become efficient vectors of malaria

Again, Sur's findings in Bengal, of the high degree of infectivity of *A philippinensis*, are in direct contrast to my own findings in Cachar In this district *A philippinensis* represents over 20 per cent of the total *Anopheles*, which have been collected and classified during the past four years This species is found breeding freely on terrain practically free from malaria, and, as I have mentioned before, it feeds chiefly in cowsheds Is zoophilism the factor in Cachar, which prevents species, which are known to be efficient carriers of malaria, in Nature elsewhere, from doing likewise in this district? Or are there other factors? In a recent address on the control of malaria in Assam, I mentioned the possibility of another factor which may have an influence on the infectivity of different species, namely the chemical or bacterial contents of the water, in which the species is found breeding This I am at present investigating by comparing the infectivity of species which have been caught, in their larval stage, in the same water as *A minimus*, and employing the infectivity index of *A minimus* as a control I am also investigating the infectivity of *A minimus*, which are being bred out in the laboratory in varying types and degrees of contaminated water, which normally would be avoided as breeding-places by this species in Nature, but which would be accepted by other species such as *A vagus, A hyrcanus, A philippinensis*, etc

According to Covell (*loc cit*) in 1919 Swellengrebel, Schuffner and S de Graaf pointed out that the following questions must be answered in assessing the capabilities of any particular species as a factor in transmission

(1) Does the species occur in great numbers, or is it rare ?

(2) Is it capable of allowing the parasites of malaria to complete their development ?

(3) Does it habitually feed on human blood, not only in captivity, but also in Nature?

(4) Does it feed in the jungle, or does it regularly visit man in or near his dwelling places ?

(5) What is the vegetable food of the females, and does it interfere with the development of the malarial parasites ?

Swellengrebel, Schuffner and de Graaf's questions can be answered, to a large extent, in Cachar, with the exception of the vegetable food of mosquitoes But is the fluid imbibed by female mosquitoes in Nature limited only to blood and vegetable juices ? Again, another question arises do female mosquitoes

imbibe water during their visitations to their breeding-places? And, if so, may not the bacterial or chemical contents, of imbibed water, affect the development of ingested gametocytes? My experience has been, that the water which forms the breeding-places of *A minimus*, in highly malarious sites, is clear, uncontaminated water.

The value of malaria infectivity surveys of Anopheline mosquitoes, caught in Nature, has lately been questioned. Recently Mayne has stated that oocysts and sporozoites in Anopheline mosquitoes, caught in Nature, may not be of the human variety. The sporozoites of bird malaria, as Mayne himself demonstrated in my laboratory, can, of course, be readily distinguished from the sporozoites of human malaria by a trained observer. Again, the findings in this survey, of mosquitoes caught in Nature, apparently have not been vitiated by the possible complication of monkey malaria, as monkeys are very rarely seen in the areas, which were being investigated.

There are still several points which have arisen during this investigation and which have not been discussed here. These I hope to refer to on another occasion, when I have had an opportunity of extending my observations.

Summary

An Anopheline infectivity survey was carried out in the Cachar district of Assam from April 1st, 1927 to March 31st, 1930.

During the survey, 42,300 adult Anopheline mosquitoes, comprising eighteen species, were caught in human habitations, cowsheds and hospitals, and dissected in the Labac Laboratory. The findings in this investigation show that *Anopheles minimus* is practically entirely responsible for the transmission of malaria in tea estates in the Cachar district of Assam.

The only two other species, found infected in Nature, were *A ramsayi* and *A hoch*, but both are of little sanitary importance compared with *A minimus*, in the Cachar district of Assam.

The other common species of Cachar Anopheline mosquitoes feed chiefly in cowsheds, and the importance of zoophilism has been stressed with such species as *A maculatus* and *A aconitus*, which have been proved to be natural carriers of malaria in countries where human blood, in the comparative absence of cattle, is practically the only blood available.

The period of the year, when mosquitoes were found infected in Nature, and the associated climatic conditions are recorded, but further research, to demarcate this period accurately, is essential.

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I also wish to record here my gratitude to the Indian Research Fund Association for partly financing this investigation from April 1st to December 31st, 1927

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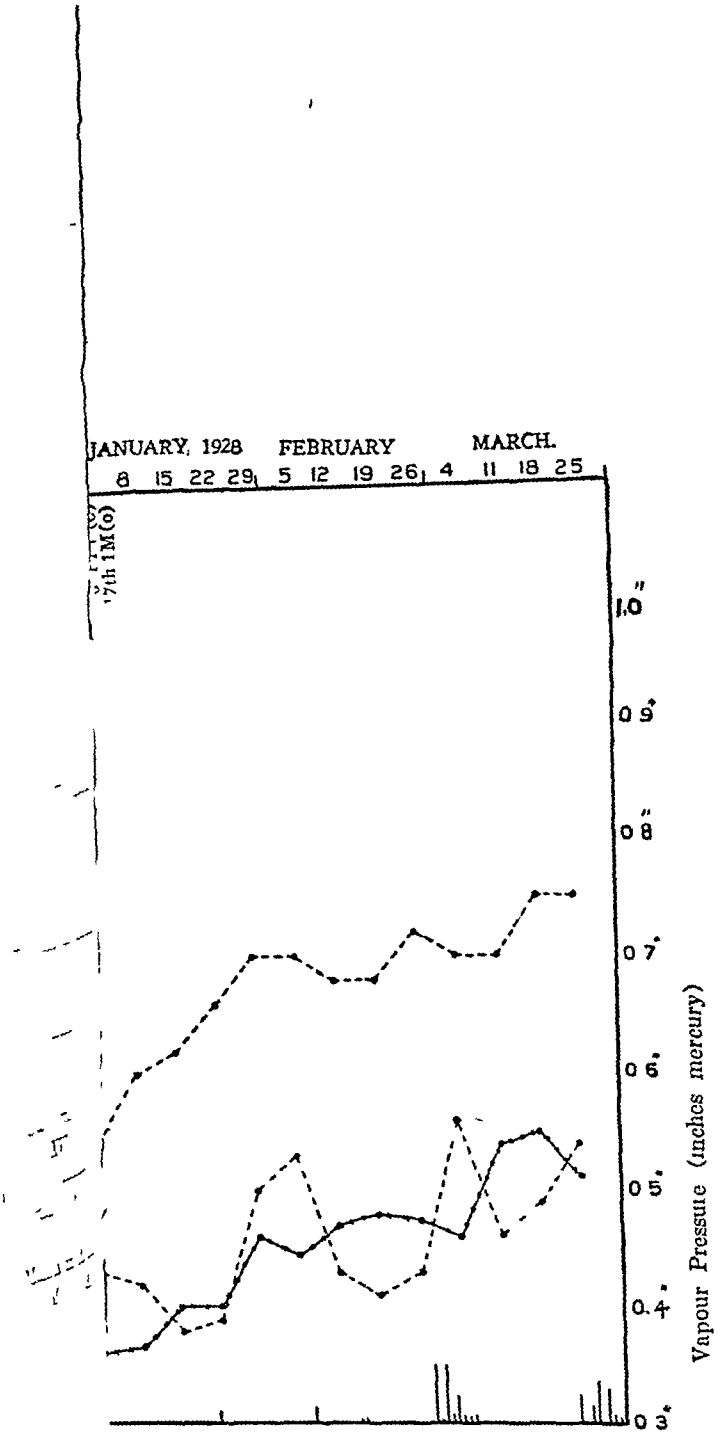
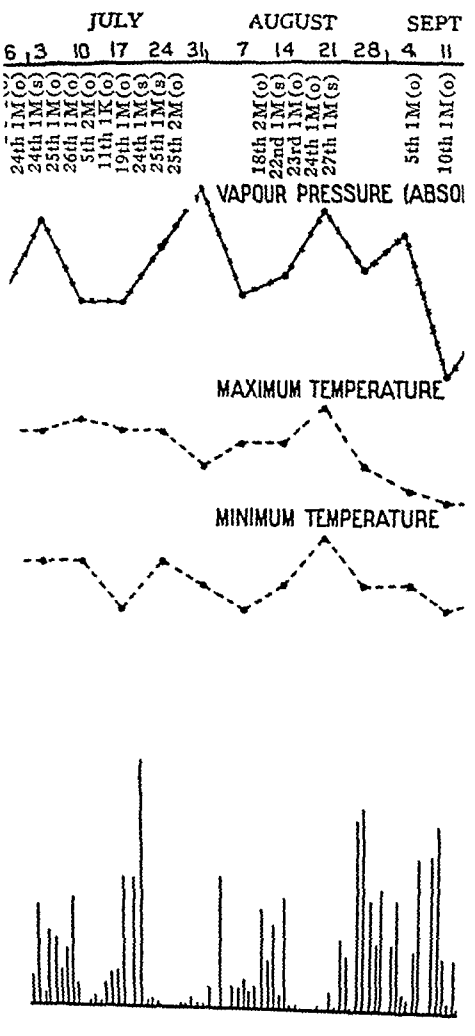


Total Rainfall 1507 inches



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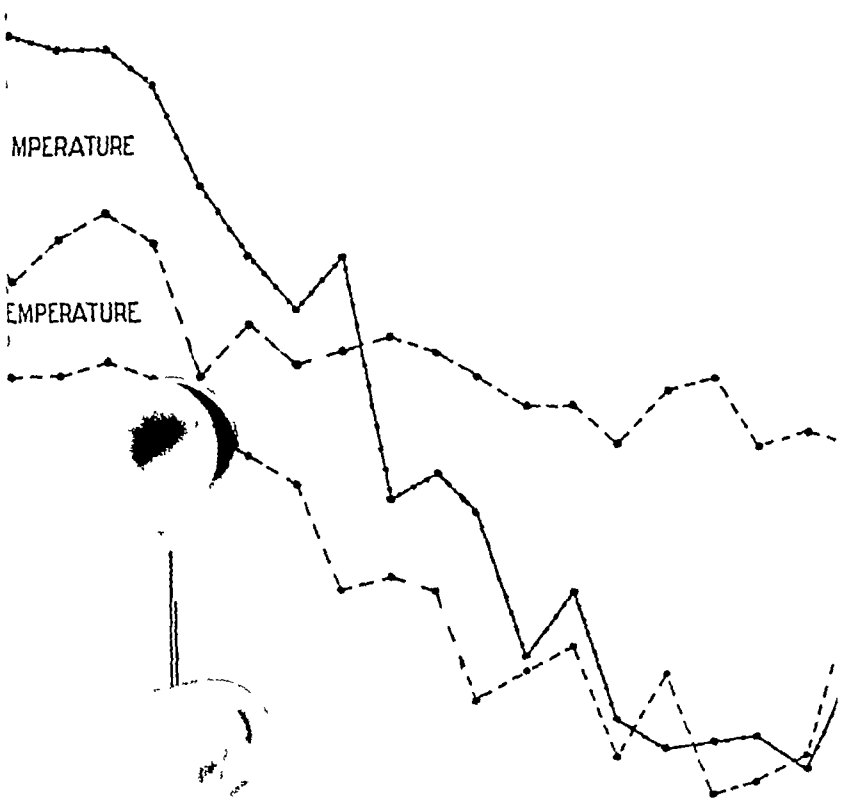
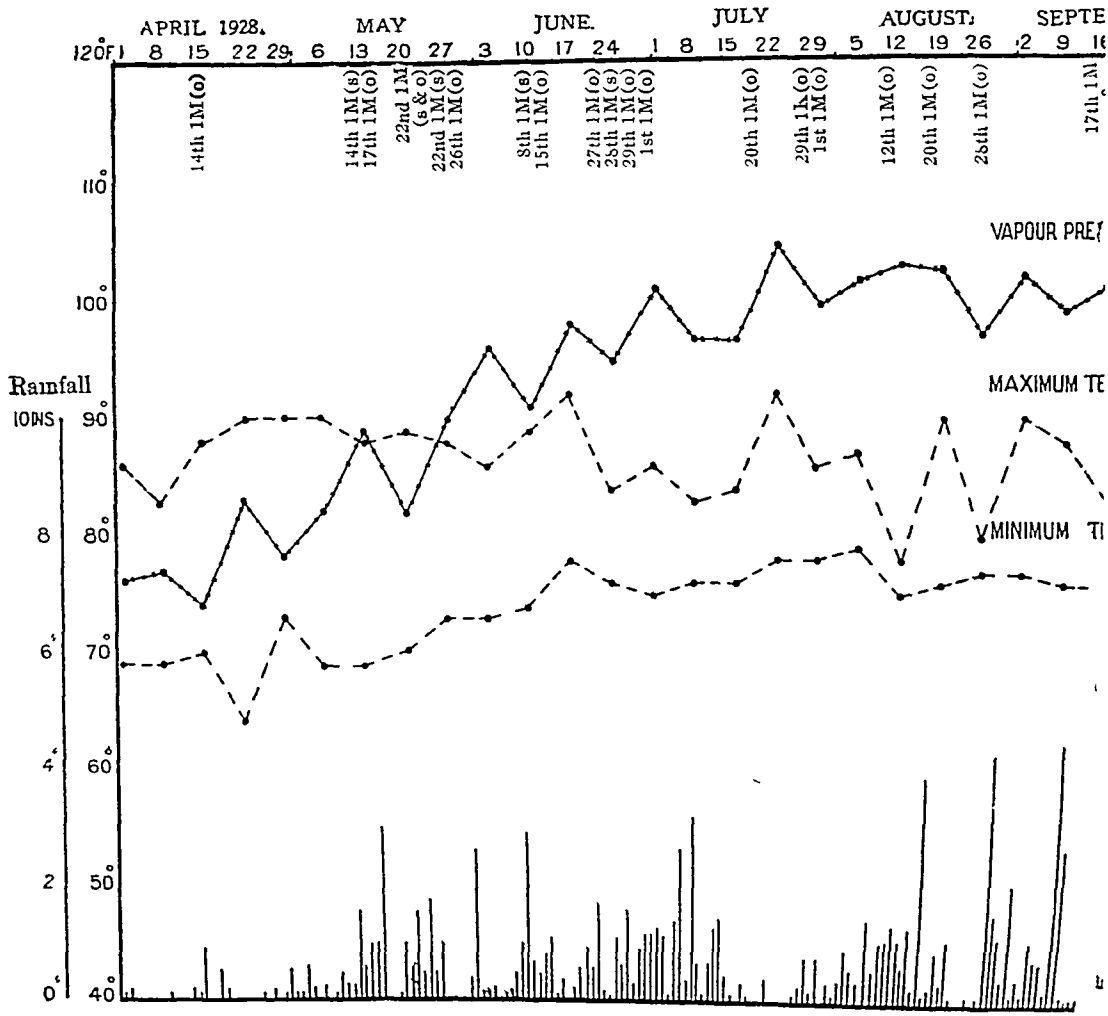
SURE (ABSOLUTE HUMIDITY)



Total Rainfall 134 51



M = *A minimus* K = *A kochi*
S = Sporozoite infection O = Oocyst infection

NOTE ON THE SIZE OF THE THYROID GLAND OF ALBINO RATS (COONOOR)

BY

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IN the course of the experimental investigation of *lymph-adenoid goitre*, now proceeding in these laboratories, it became necessary to determine the size of the thyroid gland in the stock albino rats which we employ for experimental work These rats have been bred through many generations and have become acclimatized to the altitude (6,000 feet above sea-level) From time to time new blood is introduced to prevent in-breeding A breeding stock of between 500 and 1,000 animals is maintained The health of this stock is perfect, their fertility is high During the past 18 months no case of sickness nor death from natural causes has occurred amongst them Infantile mortality is practically nil, such deaths as occur amongst very young animals are due to accidental causes The average size of the litters is eight, but occasionally litters of 12 or even 15 are born The mothers invariably rear the whole of their young The animals live under conditions of perfect hygiene, scrupulous cleanliness is maintained by a large staff of skilled animal attendants The rats are confined in roomy cages admitting of free exercise Fresh straw is used for bedding, warmth and the necessary privacy for breeding The animals are frequently to be seen nibbling the straw They are fed on a diet consisting of whole-wheat-flour *chapattis* lightly smeared with fresh butter, the hard crusts of white bread, diluted whole milk, sprouted *gram* (legume), raw carrots and cabbage *ad libitum*, and an occasional small ration of raw meat Fresh water is provided in abundance for drinking and washing purposes

During the winter of 1929-30—27th November, 1929 to 10th February, 1930—107 rats, aged between 20 and 150 days and of body-weights ranging between 27 and 226 grammes, were killed by drowning and their thyroid glands removed

At this season the maximum temperature in the animal houses varied between 73° and 53°F and the minimum between 58° and 42°F The glands were weighed immediately after removal from the body, care being taken to prevent loss of weight due to drying In these rats the isthmus of the gland is tenuous, narrow, fragile and of negligible weight, no attempt was, therefore, made to remove it with the attached lobes The latter were removed separately, and rapidly freed from adherent muscular and other tissue

The body-weights and the thyroid-weights of the 107 animals were submitted to Professor K B Madhava, who has very kindly prepared from them the following equation *Weight of thyroid gland (sexes combined) on body-weight* *Thyroid-weight in mg* = 0 0609 (*Bd wt* — 20) + 2 4210 *log* (*Bd wt* — 20) — 0 1153

From this equation the following standard table was constructed, giving the weight of the thyroid (in mg) corresponding to given body-weights This has been graphed in the figure below (p 555)

TABLE

Standard weights of the thyroid gland for given body-weights

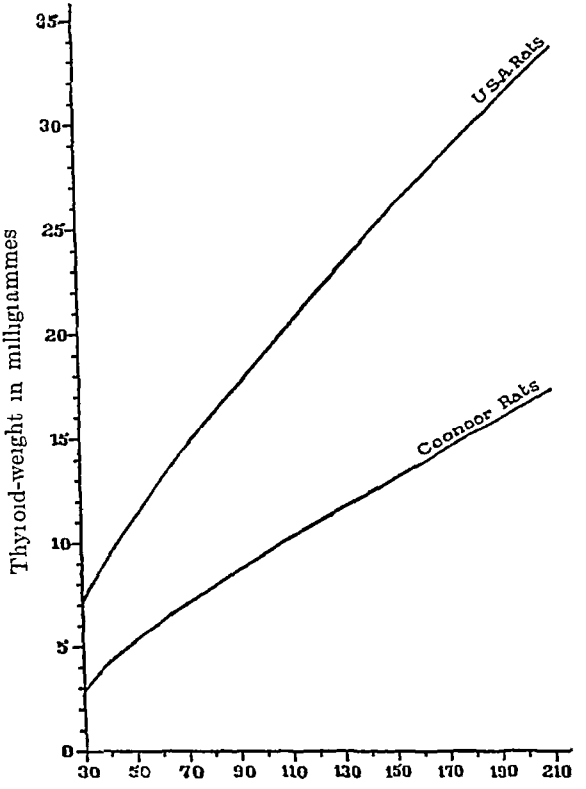
Body-weight in grammes	Thyroid-weight in mg
30	2 9
40	4 3
50	5 3
60	6 2
70	7 0
80	7 8
90	8 6
100	9 4
110	10 1
120	10 8
130	11 5
140	12 2
150	12 9
160	13 6
170	14 3
180	15 0
190	15 6
200	16 3
210	17 0

It is seen from this Table and Graph that the growth of the thyroid is relatively more rapid at lighter than at heavier body-weights (i e, in earlier than in later life)

The logarithmic curve for Coonoor rats' thyroids is shown in the Graph below in contrast with that for U S A rats' thyroids Hatai's (1913)* equation for the latter is as follows —

Weight of thyroid (sexes combined) on body-weight —Thyroid-weight in grammes = 0 0000973 (*Bd wt* + 27) + 0 0139 *log* (*Bd wt* + 27) — 0 0226

GRAPH



Showing the weight of the thyroid gland of the Coonoor albino rat, according to body-weight, in comparison with that of the U S A albino rat

The observed weights for Coonoor rats are represented by 55 males and 52 females There is no appreciable difference in the weight of the thyroid gland in the two sexes

It will be noted that at all body-weights the thyroid gland of Coonoor stock rats is approximately half the size of that of U S A rats

It is to be emphasized that the data here given relate only to 'winter glands'

The iodine-content of these glands is dealt with, by Dr G Sankaran, in a succeeding paper

* Donaldson's 'The Rat,' 1924

THE ESTIMATION OF IODINE IN THE THYROID GLANDS OF ALBINO RATS

BY

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AND

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FOR the estimation of iodine in rats' thyroids we have adopted v Fellenberg's method with certain modifications The most important of these is the lesser amount of potash added before the organic matter is destroyed by ignition Fellenberg (1924) himself recommends 1 gramme of potash for 10 to 20 mg of thyroid Leitch and Henderson (1926), in their modification, use 20 c c of 10 per cent potash (= 2 0 grammes KOH) for the same amount of thyroid We use only 2 c c of 10 per cent potash for the rat's thyroid which normally weighs 10 to 20 mg in young adult animals We have found that the lesser amount of potash is sufficient to prevent loss of iodine and that its use makes the subsequent manipulations much easier especially the ashing The rats' thyroid can be completely ashed without ever becoming red hot if this amount of potash be used

On theoretical grounds this smaller amount of potash might be expected to be sufficient A very small fraction of the iodine in the thyroid exists as inorganic iodide (Kocher, 1927), the major portion is present as a compound of thyroxine and di-iodotyrosine with a globulin (Harrington, 1929) The latter is easily hydrolysed by weak alkali, yielding amongst other products, thyroxine and di-iodotyrosine Kendal (1919) originally used 5 per cent caustic soda Harrington (1926) used 10 per cent barium hydroxide and finds that stronger alkali decomposes the thyroxine Thyroxine itself decomposes, with the liberation of iodine, somewhere about 230°C and di-iodotyrosine at a lower temperature (Harrington, 1929) It, therefore, seems unnecessary to add any great concentration of potash or to raise the temperature to red heat in order to convert the combined iodine into iodide

Ashing

The whole of the thyroid gland, carefully dissected out and freed from adherent muscle and other extraneous tissues, is accurately weighed at once (ordinary weight 10 to 20 mg.), and dropped into a small test-tube containing 2 c.c. of 10 per cent. potassium hydroxide solution. Once in the potash it can be left for days or weeks, if properly corked or sealed off, until it is convenient to proceed with the rest of the analysis. The thyroid dissolves more or less completely—generally in the cold but more readily on warming. The solution is transferred to a nickel crucible and slowly evaporated to dryness. With care there need be no frothing nor spurting. The residue is then gently roasted over a naked flame with stirring, care being taken that no part becomes red-hot. With the small amount of potash added it is possible to get a grey, powdery ash under these conditions. The ash is then dissolved in a few drops of water and evaporated until a viscous paste is obtained. This is now ready for alcoholic extraction.

Alcoholic extraction

Two extractions are made with an ignition of the extract between them. The first is done with six lots of alcohol—first 0·3 c.c. and subsequently 0·1 c.c. lots—and the combined extracts poured into a nickel (not platinum) dish. Eighty per cent. alcohol is used unless the pasty mass becomes too fluid and tends to run over with the alcohol. When this happens 95 per cent. alcohol is used for one of the lots and this has the effect of restoring the pasty mass to its proper consistency.*

After extraction, one drop of ten per cent. potash is added to the extract which is then evaporated to dryness and heated to below redness over a naked flame. The amount of residue is very small and charring seldom occurs.

The second extraction is made in the same way as the first except that the extract is collected in a platinum dish. One drop of one per cent. potash is added and the extract evaporated to dryness on a water-bath, the dish is then waved over a naked flame. The residue is now hardly visible.

The colorimetric estimation

The residue is now dissolved in water and poured into a Fellenberg's tube. In all 12 drops of water are used, first 3 drops and afterwards 1 or 2 drops at a time making the total bulk up to less than 0·5 c.c. One drop of 5 N sulphuric acid and one drop of N/10 sodium arsenite are then added and the whole diluted to exactly 0·5 c.c. After half an hour one drop of carbon disulphide and one drop of nitrose are added, and the tube shaken, centrifuged, and compared with standards in the usual way.

Control analyses.

For the purpose of testing the accuracy of the method sheep's thyroid was used to provide a large amount of test material from which uniform samples

* A nickel dish should be used for collecting the first extract, if a platinum one be used, a dark stain appears during the subsequent heating which is difficult to remove.

could be drawn Three grammes of fresh sheep's thyroid were mixed with 25 c c of 10 per cent potash After some days solution was nearly complete, the few remaining floccules of undissolved matter were filtered off, and the clear solution diluted first, so that one litre corresponded to 30 grammes of thyroid, and afterwards, so that 1 litre corresponded to 1 5 grammes of fresh thyroid It was found that the sheep's thyroid was much richer in iodine than rat's thyroid, the higher dilution of the sheep's thyroid-solution was necessary so that a convenient quantity (0 5–1 0 c c) should yield a suitable amount of iodine for colorimetric comparison (1 to 2γ)

Using varying quantities of this stock thyroid-solution, with or without iodine in the form of potassium iodide, 18 test analyses were made The results are shown in the following Table —

TABLE

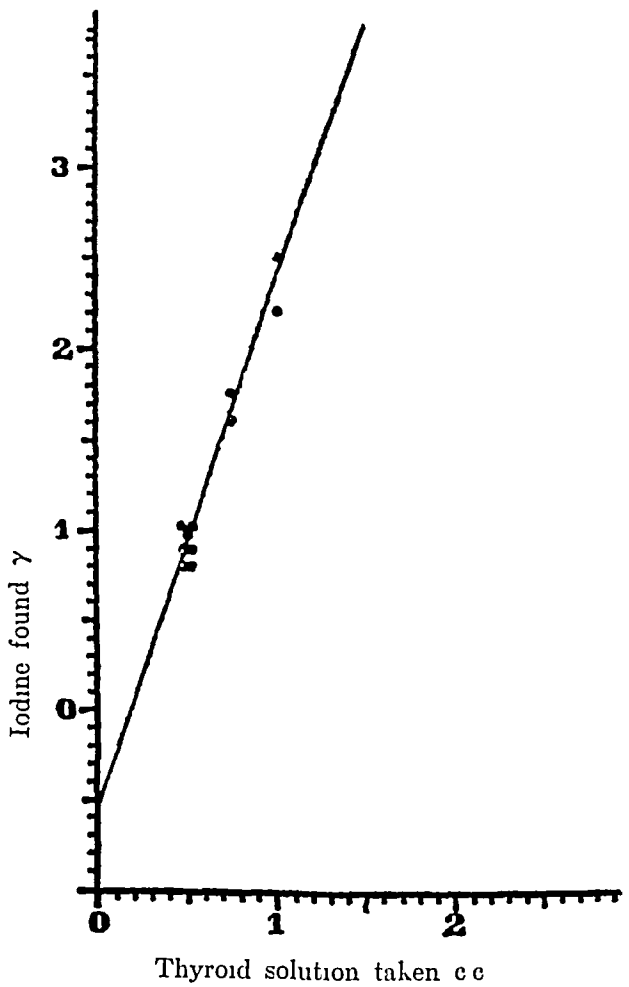
Showing the results of control analyses

Volume of thyroid solution taken in c c	Iodine added in γ	Iodine found in γ	
0·5	0 0	1 0	
0·5	0·0	0·8	
0·5	0·0	0 9	
0·5	0·0	0·8	Mean 0·9
0·5	0·0	1 0	
0·5	0·0	1 0	
0·5	0·0	0·9	
0·75	0·0	1 75	Mean 1 7
0·75	0·0	1 6	
1 0	0·0	2·5	Mean 2 4
1 0	0·0	2·2	
0 5	1 0	2 0	
0 5	1 0	1 8	
0 5	1 0	1 6	Mean 1 7
0 5	1 0	1 5	
0 5	1 0	1 5	
0 5	2·0	2·5	Mean 2 7
0 5	2·0	2·8	

Considering first the eleven control analyses in which no iodine was added it the quantities of thyroid solution taken for analysis are plotted against the

amount (γ) of iodine found, then (*vide* Diagram) the results lie on a nearly straight line, and quite as close to it as might be expected from the known errors of the estimation (e g , 20 per cent error in the colorimetric estimation) This line, however, does not cut the vertical axis at 0 but at 0 5 and from this one must conclude that there is a constant loss of 0 5 γ of iodine in the process, and that this loss is the same whatever be the quantity of solution, between 0 5 and 1 0 c c (corresponding to 0 9 to 2 1 γ of iodine), taken for analysis

DIAGRAM



Considering next the 14 control analyses in which 0, 1 and 2 γ of iodine were added to 0 5 c c of thyroid solution, we have the following results —

0 γ added	0 9 γ iodine found
1 γ do	1 7 γ do
2 γ do	2 7 γ do

the differences being 0 8 and 1 0 or on the average 0 9 γ This indicates that of the iodine (up to 2 γ) added as KI an average of 90 per cent is recovered, or within the expected errors of the method, the whole of it

The method of iodine-estimation here outlined would thus appear to be of sufficient accuracy It was employed by one of us (Sankaran, 1930) in the investigation with which the succeeding paper deals

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THE IODINE-CONTENT OF THE NORMAL THYROID OF ALBINO RATS

BY

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(From the Nutritional Research Laboratories, I R F A, Pasteur Institute, Coonoor)

[Received for publication, June 4, 1930]

Introduction

THE records (Orr and Leitch, 1929) in the literature of estimations of the iodine-content of the normal thyroid gland of the albino rat are relatively few Different workers have given figures which vary very much, and it seems likely that the iodine-content of the normal gland may differ in different parts of the world

The present paper deals with the iodine-content of the 107 Coonoor stock, albino rats referred to in the foregoing note by McCarrison (1930, page 553) On removal of the glands they were weighed and handed to me for assay of their iodine-content My sole information regarding them was their serial number Not until the estimations of their iodine-content had been completed was I provided with details regarding the body-weight and sex of the rats from which they were removed

There were in all 107 glands 55 males and 52 females Details regarding them are set out in Table I It is to be emphasized that the results recorded in this paper relate to mid-winter glands only

Method of iodine estimation and results

The method of iodine-estimation was that described in a previous paper (Newcomb and Sankaran, 1930, page 557), it is a modification of v Fellenberg's

The results are set out in Table I Certain statistics calculated from them are given in Table II These tables will be found at the end of the paper

For a better understanding of the figures three diagrams have been made showing the relations between (*a*) body-weight and thyroid-weight, (*b*) thyroid-weight and total iodine-content of the thyroid, and (*c*) body-weight and total iodine-content of thyroids

It was found that the iodine-content of rat thyroids was, in this locality, very low, when compared with the iodine-content of the thyroids of various other species of animals

TABLE

Showing the iodine-content of thyroids of animals found by other workers

Animal	Iodine-content in grammes per 100 gs Fresh weight	Authority
Sheep	0·026 to 0·038	Baumann (1896.2)
Do	0·011 to 0·156	Do
Do	0·031 to 0·035	Do
Cattle (normal)	0·112	Marine and Lenhart (1909·1)
Sheep (normal)	0·069	Do
Hog (normal)	0·088	Do
Sheep (normal)	0·096	Fenger (1913)
Hog	0·041 to 0·051	Aldrich (1915)
Rabbits	0·023 to 0·05	Rowett Institute (unpublished)
Fowl	0·14 to 0·209	Do
Our figures for rats	0·007 to 0·042	1930

Our figures for rats (0·007 to 0·042 per cent of fresh gland, mean 0·018) are, however, higher than those given by other observers Hercus and Roberts give values for the iodine-content of the fresh gland which range between 0·003 and 0·007 per cent In an unpublished work from the Rowett Institute (Orr and Leitch, 1929) a figure 0·007 to 0·037 per cent is given Our lowest value is the same as the highest of Hercus and Roberts, while our highest is higher than the highest value of the Aberdeen workers

The moisture-content of three of the thyroids was determined The method employed was to put the weighed glands into glass tubes of less than a gramme in weight and then to place the tubes over calcium chloride in a vacuum desiccator After a week's time the glands were found to be completely dry, there

being no further loss of weight on keeping the tubes containing them in a hot air oven for 4 hours at 100° to 110°C The results are given below —

Thyroid No	109	Moisture per cent	78 5
Do	110	„ „	77 0
Do	112	„ „	80 0

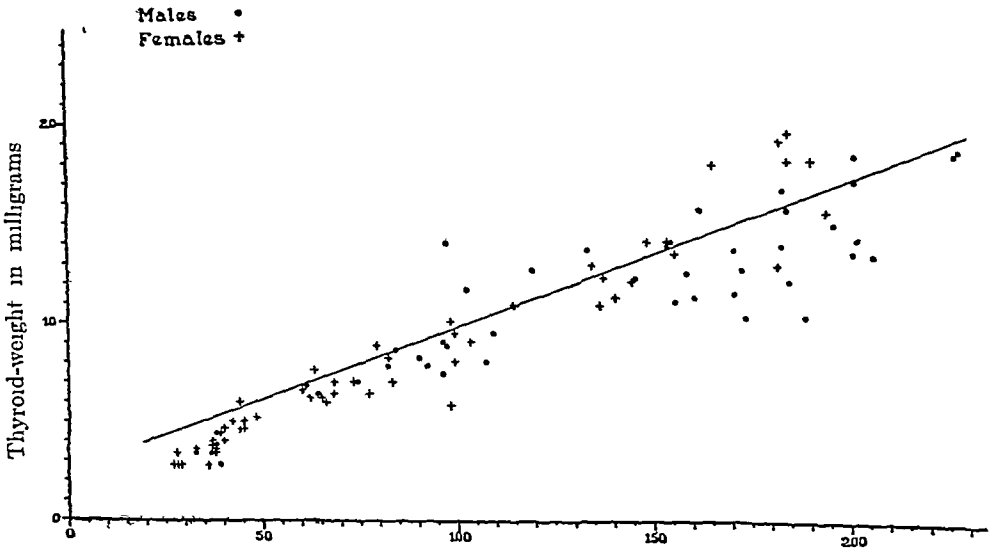
The iodine-content of these glands were determined and was found to fit in with the thyroid-weight-iodine-content curve This indicates that there is no loss of iodine on desiccation at 110°C

Statistical analysis of the results.

The statistical analysis of the results is summarized under several headings, as follows —

(*a*) *Body-weight and thyroid-weight* —Diagram 1 shows the relationship between the body-weights and the thyroid-weights of these rats This relation-

DIAGRAM 1



ship can be adequately represented by a straight line which fits the observations closely enough for my present purpose * The equation of this line is —

$$Y = 0\ 072\ X + 2\ 69$$

where Y is the thyroid-weight in milligrams, and X is the body-weight in grammes The line has been calculated for all the rats, both male and female, there being no certainly demonstrable difference between males and females

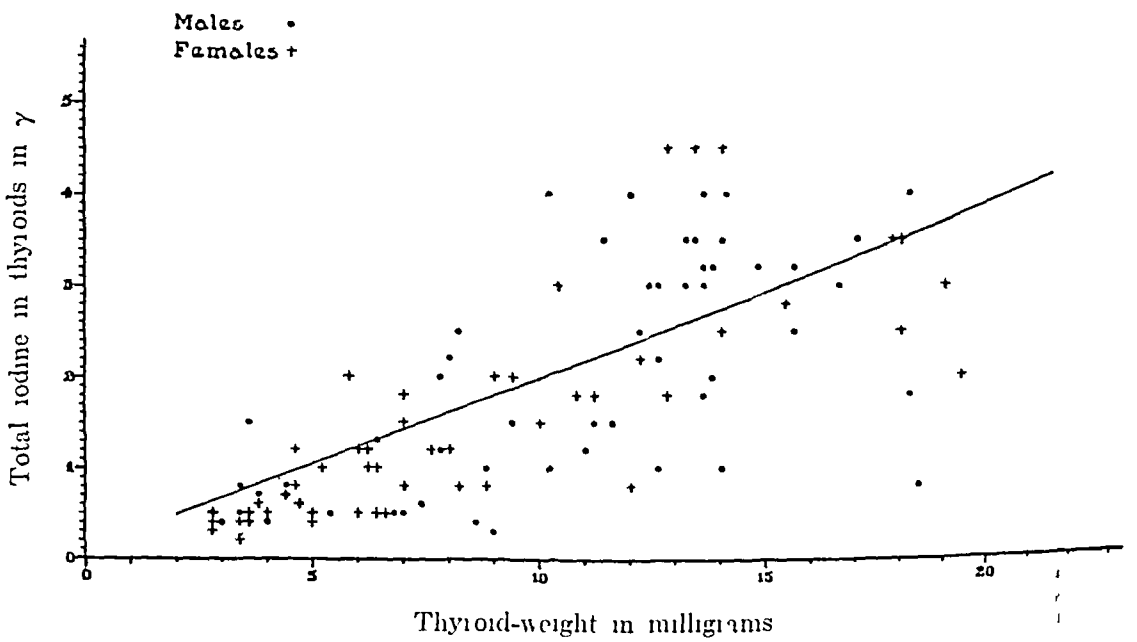
*For logarithmic curve and formula for the same, *see* previous note by McCarrison (1930, page 553)

(*b*) *Thyroid-weight and iodine-content*.—A statistical consideration of the total iodine-content of the thyroid in relation to the thyroid-weight indicates that the two are proportional, and that there is no significant difference between the sexes. The observations are adequately fitted by straight regression lines and these are given by the equations:—

Males	$Z = 0\ 186Y - 0\ 00$
Females	$Z = 0\ 178Y - 0\ 01$
All observations	$Z = 0\ 197Y - 0\ 09$

where Y = thyroid-weight and Z = iodine-content. The line drawn in Diagram 2 is the one for all the observations.

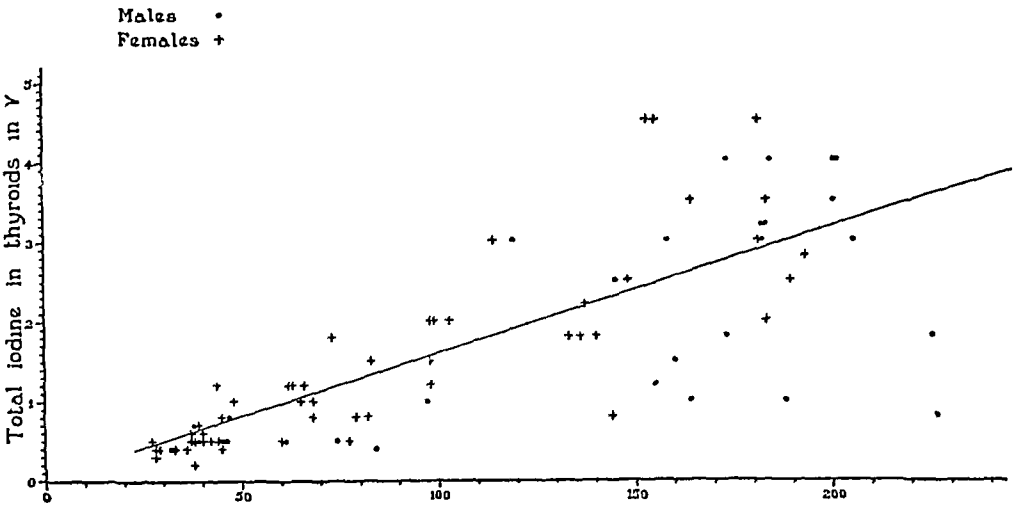
DIAGRAM 2



The mean thyroid-weight is 9 44 mg. and the mean total iodine-content of the thyroids is 1 71 γ. There is, therefore, on the average $\frac{1\ 71}{9\ 44} = 0\ 182\ \gamma$ of iodine per milligram of fresh thyroid, i.e., 0 0182 per cent. of iodine.

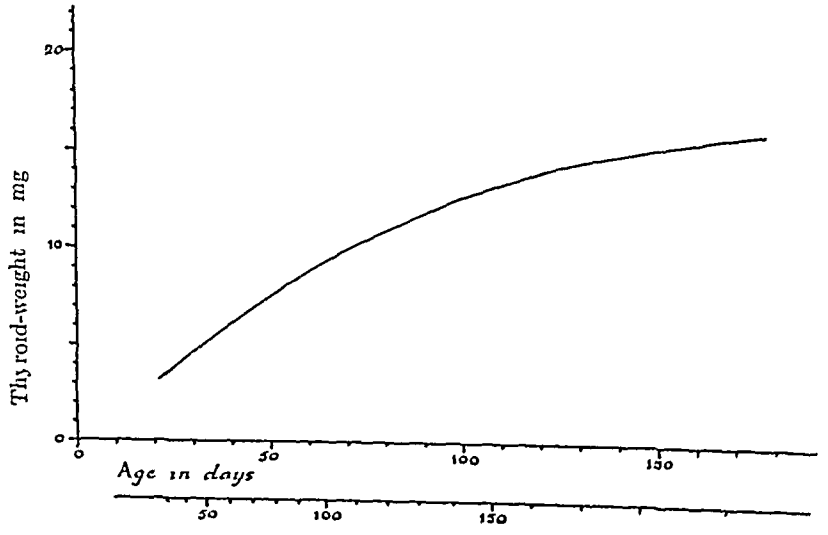
(*c*) *Body-weight and iodine-content of thyroids*.—As the thyroid-weights are nearly proportional to body-weight and as the iodine-content is proportional to thyroid-weight, there must be an almost direct proportion between the total iodine in the thyroids and the body-weights. Diagram 3 shows this relationship. The regression equation for both sexes together is $Z = 0\ 0158\ X + 0\ 01$, where Z = iodine-content and X = body-weight. As the mean body-weight is 107 6 grammes and the mean iodine-content of the thyroids is 1 71 γ, it follows that there is on the average $\frac{1\ 71}{107\ 6} = 1\ 59\ \gamma$ iodine in the thyroid gland per 100 grammes body-weight.

DIAGRAM 3



(*d*) *Age*—The ages of these rats were not definitely known though their approximate ages ranged between 20 and 150 days The relation of body-weight to age in our stock rats has, however, been previously determined so that the ages of these rats can be deduced from their body-weights, though the

DIAGRAM 4



error for a particular rat may be large The body-weights which, in our stock rats, correspond to ages from 50 to 100 days—the age of puberty—are 78 to 143 grammes There is no indication of a rise in the total iodine in the thyroid between the body-weights which would correspond to the attainment to sexual maturity Rats below 27 grammes in weight (corresponding to 20 days of age) have not been studied, and it is possible that at this age-period a sudden change in the rate of increase may occur Diagram 1 illustrates the relation of thyroid-weight to age, deduced from the curve found for thyroid-weight on body-weight and the curve previously found for the regression of body-weight on age for our stock rats

(*e*) *Sex*.—An examination of the figures for iodine per *milligram* of thyroid revealed no sex difference which could be regarded as statistically significant

Comparison of results with those of previous observers

(1) From a study of thyroids of cattle v Fellenberg (1927) came to the conclusion that the iodine-content increases with increasing weight of the thyroids, but adds that there is no strict proportion Arnold and Gley (1923) found no relation between the iodine-content and the weight of the gland in goats It is surprising, therefore, that older workers found an inverse relationship between the size of the gland and its iodine-content Bauman noted it in man and sheep, so also did Marine and Lenhart, Martin and Aldrich noted it in the thyroids of sheep, oxen and pigs Martin's figures are given below —

Fresh weight of gland	Iodine
120 gs	0·22 gs
140 „	0·2 „
325 „	0·08 „

(2) In the older work of Bauman, Oswald, and Moneiy, no difference was noted in the iodine-content of the thyroid gland due to sex and this is also Choudhury's (1928) conclusion Fenger claims to have detected a sex-difference in the iodine-content from an examination of 17 male and 23 female calves—castrated animals resembling the females The figures given by him are 0·21 per cent for males and 0·25 per cent for females, the percentages

being on a dry fat-free basis Unless the variation in these experiments was very much smaller than that in ours the figures are, statistically speaking, not significantly different Fenger, however, in discussing the iodine-content according to age gives the figures for several age-periods as 0 35 per cent, 0 23 per cent, 0 35 per cent and 0 28 per cent There is here a variation much greater than what he observed for sex-difference, yet he says there was no marked difference due to age

Limitations of this study

The subject of the iodine-content of the albino rat's thyroid is not reviewed fully in this short note For instance, the thyroids of rats from birth or from intra-uterine life up to 27 grammes body-weight have not been studied, such a study may reveal some important results At the other end of the life-cycle it may be of interest to know the iodine-content of old and senile rat thyroids up to the point of death from senility

The observations recorded in this paper were made in the winter months Many observers have noted seasonal variations in the iodine-content of the thyroid gland, it is hoped, therefore, to carry out a similar investigation during the summer months

With the limitations noted it is hoped that the number of estimations (107), being greater than any previously done, will give a fairer representation of the figure for this particular animal The main interest of the work for this laboratory is that it enables us to compare the iodine-content of the normal thyroid of our albino rats with that of the goitres produced under experimental conditions by Colonel McCarrison

Summary and conclusions

Figures are given for weights and iodine-content of the thyroids of 107 normal Coonoor stock, albino rats The relation of the iodine-content of the thyroid to thyroid-weight and to body-weight has been studied statistically —

(1) The thyroid-weight increases with the body-weight The two are almost directly proportional and the relationship has been represented by a regression line, there is no demonstrable difference between the two sexes

(2) The iodine-content of the thyroids is directly proportional to their weight and has a mean value of 0 018 per cent

(3) Sex and age make no difference in the concentration of iodine in the thyroid

My thanks are due to Lieut-Colonel C Newcomb, I M S, for working out the statistical figures and for Diagram 4

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McCarrison (1930)	*Ind Jour Med Res*, XVIII, 2, p 553

TABLE I

1	2	3	4	5	6
Serial number	Date of killing	Body-weight grammes	Sex	Thyroid-weight mg	Total iodine in thyroid
1	27-11-29	44	F	4·6	1·2
2	„	44	„	6·0	0·5
3	„	45	„	4·6	0·8
4	„	48	„	5·2	1·0
5	„	45	„	5·0	0·4
6	„	42	„	5·0	0·5
7	„	47	M	4·4	0·8
8	„	42	„	3·6	1·5
9	„	45	„	5·0	0·5
10	„	46	„	5·4	0·5
11	„	82	„	7·8	1·2
12	„	64	„	6·4	1·3
13	28-11-29	77	F	6·4	0·5
14	„	84	M	8·6	0·4
15	„	99	F	9·4	2·0
16	„	90	M	8·2	2·5
17	„	98	„	10·0	1·5
18	„	92	„	7·8	2·0
19	„	66	F	6·0	1·2
20	„	102	M	11·6	1·5
21	„	82	F	8·2	0·8
22	„	109	M	9·4	1·5
23	„	103	F	9·0	2·0
24	„	107	M	8·0	2·2
25	29-11-29	144	F	12·0	0·8
26	„	161	M	15·6	2·5
27	„	181	F	19·0	3·0
28	„	153	M	13·0	2·0

TABLE I—*contd*

1	2	3	4	5	6
Serial number	Date of killing	Body-weight grammes	Sex	Thyroid-weight mg	Total iodine in thyroid
29	29-11-29	164	F	17·8	3·5
30	"	155	M	11·0	1·2
31	"	114	F	10·4	3·0
32	"	164	M	12·6	1·0
33	"	98	F	8·0	1·2
34	"	173	M	13·6	1·8
35	"	133	F	12·8	1·8
36	"	115	M	12·2	2·5
37	30-11-29	184	"	12·0	1·0
38	"	151	"	11·0	3·5
39	"	205	"	13·2	1·0
40	"	97	"	8·8	1·0
41	"	225	"	18·2	1·8
42	"	226	"	18·4	0·8
43	"	200	"	13·4	3·5
44	"	182	"	16·6	3·0
45	"	201	"	11·1	4·0
46	"	173	"	10·2	4·0
47	"	170	"	11·4	3·5
48	"	174	"	13·2	3·5
49	"	182	"	13·8	3·2
50	"	119	"	12·6	3·0
51	"	188	"	10·2	1·0
52	"	160	"	11·2	1·5
53	"	195	"	14·8	3·2
54	"	170	"	13·6	4·0
55	"	131	"	13·6	3·0
56	3-1-30	83	F	7·0	1·5

TABLE I—*contd*

1	2	3	4	5	6
Serial number	Date of killing	Body-weight grammes	Sex	Thyroid-weight mg	Total iodine in thyroid
57	3-1-30	73	F	7 0	1 8
58	„	98	„	5 8	2·0
59	„	79	„	8·8	0·8
60	„	68	„	6 4	1 0
61		65	„	6 2	1 0
62	„	60	„	6·6	0·5
63	3-10-30	68	„	7 0	0·8
64	„	63	„	7 6	1·2
65	„	62	„	6·2	1·2
66	„	97	M	14·0	1 0
67	„	96	„	9 0	0·3
68	„	96	„	7 4	0 6
69	,	74	,	7 0	0·5
70	„	61	,	6·8	0·5
71	6-1-30	200	„	17 0	3 5
72		172	,	12·6	2·2
73	,	200	,	18·2	4 0
74		183	„	15 6	3 2
75		189	„	18·0	2·5
76		181	„	12 8	4 5
77		183	„	18·0	3·5
78	„	193	„	15 4	2·8
79	„	183	,	19·4	2·0
80	7-1-30	140	,	11·2	1·8
81	,	136	„	10 8	1·8
82	„	148	„	14 0	2·5

TABLE I—*concld*

1	2	3	4	5	6
Serial number	Date of killing	Body-weight grammes	Sex	Thyroid-weight mg	Total iodine in thyroid
83	7-1-30	137	M	12·2	2·2
84	,,	153	,,	11·0	4·5
85	,,	155		13·1	4·5
86	,,	158		12·1	3·0
87	,,	133		13·6	3·2
88	10-2-30	37		[illegible]	0·5
89	,,	36		[illegible]	0·1
90	,,	37		[illegible]	0·6
91	,,	37		[illegible]	0·5
92	,,	33	F	3·6	0·4
93		29	,,	2·8	0·4
94	,,	33	M	1·0	[illegible]
95	,,	39	F	1·[illegible]	0·7
96	,,	38	,,	3·6	0·5
97	,,	40	,,	1·7	0·6
98	,,	39	M	2·8	0·5
99	,,	33	,,	3·1	0·8
100	,,	38		4	0·7
101	,,	40			0·5
102	,,	38			0·2
103	,,	38			0·7
104	,,				0·4
105	,,				
106	,,				
107	,,				

TABLE II

Statistic	Males	Females	All
Number	55	52	107
Mean body-weight in grammes	126 0	88 4	107 66
„ thyroid-weight in milligrams	10·47	8·36	9 44
„ iodine-content in γ	1·95	1 45	1 71
Standard deviation of			
body-weight	60 6	53 2	60·4
thyroid-weight	4 46	4 88	4 7
iodine-content	1 26	0·94	1·24
Correlation of coefficient of body-weight and thyroid-weight	0·855	0·95	0 925
„ „ body-weight and iodine-content	0·71	0·91	0·77
„ „ thyroid-weight and iodine-content	0·66	0·93	0·72

FURTHER RESEARCHES ON LYMPH-ADENOID GOITRE IN RATS

Part IV

BY

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My last report (McCarrison, 1929) on this subject dealt with experiments in which 30 young rats were fed on a diet composed of white bread 97 parts, dried yeast 3 parts, and water. Amongst these there were three cases of lymph-adenoid goitre. The 30 animals were divided into two batches, of which one—consisting of 12—was fed on the experimental diet during the spring and summer of 1928, and the other—consisting of 18—during the autumn and winter of 1928-29. The two groups were considered together, although it had been noted that all three cases of lymph-adenoid goitre occurred during the spring and summer months. As there was the possibility that this might have been due to chance, no reference was made to the suspected seasonal incidence of the condition pending a repetition of the experiment on a sufficiently large scale. This has now been done, and one purpose of the present report is to record the results.

A further subject of inquiry was the effect of lime and of iodine in favouring or disfavouring the development of lymph-adenoid goitre. It had previously (McCarrison, 1925) been observed that the addition of slaked lime to the diets of well-fed rats and pigeons had the effect of causing colloid material to accumulate in the vesicles of the thyroid gland with resultant slight, but significant, increase in size of the organ, the further addition of iodine prevented this effect of lime (McCarrison, 1925). Among the faults of the diets used in the present experiments deficiency of fat-soluble vitamins and of phosphates was conspicuous. In the presence of these deficiencies calcium-metabolism is profoundly disturbed, and when to them is added an excess of lime in the diet the excretion of calcium by way of the urinary tract is greatly

increased It seemed probable, therefore, that the effects of lime on the thyroid gland of rats fed on diets having these deficiencies might differ from those previously observed in well-fed rats

The diets hitherto used in the experimental production of lymph-adenoid goitre (McCarrison, 1927, 1928, 1929) have had two main faults in common (*a*) deficiency of fat-soluble vitamins, and (*b*) deficiency of vitamin C Certain experiments were accordingly undertaken with the object of determining which of these deficiencies was the dominant one A third purpose of the present paper is to record the results of such of these experiments as have been completed

This report deals, therefore, with the following matters —

A Confirmation of the experimental production of lymph-adenoid goitre in rats by means of a white-bread-and-yeast diet
B Influence of season on the incidence of lymph-adenoid goitre in rats
C Influence of lime on the development of lymph-adenoid goitre in rats
D Influence of iodine on the development of lymph-adenoid goitre in rats
E Relation of vitamin C-deficiency to the development of lymph-adenoid goitre in rats
F Relation of vitamin D-deficiency to the development of lymph-adenoid goitre in rats

Before proceeding to describe the experiments dealing with these matters it is necessary to emphasize, as was done in previous reports (McCarrison, 1927, 1928, 1929), certain experimental details (*a*) the experiments were carried out at an altitude of 6,000 feet above sea-level, in a locality (Coonoor) where goitre is not endemic and where the iodine-content of the soil is relatively high, (*b*) the young rats used in the experiments had no hereditary predisposition to 'goitre,' having been taken from the non-goitrous stock of these laboratories, (*c*) the greatest care was taken to exclude the goitrogenic influence of 'dirt' (McCarrison, 1930*a*), (*d*) each animal was confined in a separate, screened cage, and was abundantly supplied with water One factor alone could not be controlled infection, carried either by the rats themselves or by the lice with which they were infested

A. Confirmation of the experimental production of lymph-adenoid goitre by diets of white bread and dried yeast.

The experimental diets

Diet 1 —This consisted of white bread 97 parts, dried yeast 3 parts, and distilled water *ad libitum*

Thirty-six young rats were fed on this diet 24 during the spring and summer of 1929, and 12 during the autumn and winter of 1929-30

Diet 2—This consisted of white bread 97 parts, dried yeast 3 parts, and distilled water *ad libitum* To each 100 grammes of the white-bread-and-yeast mixture 25 grains of slaked lime were added The daily food consumption by the rats fed on this diet was approximately 12 grammes, so that each animal ingested about 3 grains of slaked lime daily

Twenty-four young rats were fed on this diet 12 during the spring and summer of 1929, and 12 during the autumn and winter of 1929-30

Diet 3—This consisted of white bread 97 parts, dried yeast 3 parts, and distilled water *ad libitum* To each 100 grammes of the white-bread-and-yeast mixture 25 drops of an iodine-solution were added This solution contained 1 0 mg (1,000 γ) of iodine per litre of distilled water The iodine provided by this addition was approximately 0 2γ per rat per day Twelve young rats were fed on this diet during the autumn and winter of 1929-30

Diet 4—This consisted of white bread 97 parts, dried yeast 3 parts, and distilled water *ad libitum* To each 100 grammes of the white-bread-and-yeast mixture 25 grains of slaked lime and 25 drops of the above iodine-solution were added

Twenty-four young rats were fed on this diet 12 during the spring and summer of 1929, and 12 during the autumn and winter of 1929-30

The average urinary excretion of iodine by the rats fed on the four diets was as follows —Diet 1, 75 γ per litre, diet 2, 85 γ per litre, diet 3, 112 γ per litre, diet 4, 122 γ per litre

The experiments and their results

The experiments fall into two categories according to the season of the year during which they were carried out —

First series Spring and summer experiments

These included (1) 24 young rats fed on diet 1 (white bread, dried yeast and water)

(2) 12 young rats were fed on diet 2 (white bread, dried yeast, lime and water)

(3) 12 young rats fed on diet 4 (white bread, dried yeast, lime, iodine and water)

The results are set out in Tables I, II and III

From an examination of these tables it is seen that there were 15 'goitres' amongst the 48 animals, an incidence of 31 2 per cent Of these, 5 occurred

TABLE 1

Giving details of the experiment in which 24 young rats were fed during the spring and summer months on diet 1, white bread, yeast and water

Number of animal	Sex	Original body-weight grammes	Maximum body-weight attained grammes	Final body-weight grammes	Days under experiment	Cause of death	Thyroid at post-mortem	Histological appearances of thyroid
2435	M	53	112	88	63	?	Small goitre	Colloid gland large cysts both lobes vessels engorged marked reticulo-endothelial reaction pale staining epithelium
2436	F	54	110	84	96	Urinary calculus	No goitre	
2437	F	57	78	65	67	Asthenia	Slight goitre	*Early lymph-adenoid goitre*
2438	F	56	91	66	77	Enteritis abscess	No goitre	Colloid gland
2439	M	52	72	48	26	Pneumonia	Do	
2440	M	52	156	97	137	Enteritis	Do	
2441	F	51	72	50	135	Inanition	Do	Colloid gland p.m. changes marked
2442	F	50	72	52	37	Bi pneumonia	Do	Exhausted gland
2443	F	56	85	58	60	Pneumonia	Do	Colloid gland congestion
2444	M	50	113	79	106	Enteritis	Slight uni lateral goitre	Colloid gland cyst in enlarged lobe vessels engorged diffuse reticulo-endothelial reaction pale staining epithelium
2445	M	60	82	57	33	Inanition	No goitre	Exhausted gland

2346	M	53	109	90	94	Urinary calculus abscess	Do	
2345	F	48	141	102	40	Pneumonia	Do	Colloid gland small cysts
2346	M	48	80	68	59	Do	Do	Do
2347	F	50	92	73	82	Do	Do	Colloid gland congestion
2348	M	47	202	142	187	Intestinal obstruction	Do	
2349	F	47	107	88	96	?	Slight goitre	Colloid gland marked congestion
2350	F	48	90	67	70	Pneumonia	Do	Colloid gland cysts both lobes vessels engorged diffuse reticulo-endothelial reaction pale staining epithelium
2351	F	48	113	92	98	Urinary calculus	No goitre	Colloid gland congestion
2352	M	46	95	80	80	Gastro-intestinal stasis	Do	Do
2353	F	45	67	58	45	Pneumonia	Do	Colloid gland p m changes marked
2354	M	50	69	69	21	Asthenia	Do	
2355	F	47	82	64	60	Enteritis, ulcer stomach	Small goitre	*Early lymph-adenoid goitre*
2356	M	46	126	85	104	Enteritis	No goitre	Colloid gland vessels engorged p m changes marked
		Average days under experiment =			78·9			

TABLE II

Giving details of the experiment in which 12 young rats were fed during the spring and summer months on diet 2, white bread, yeast, lime and water

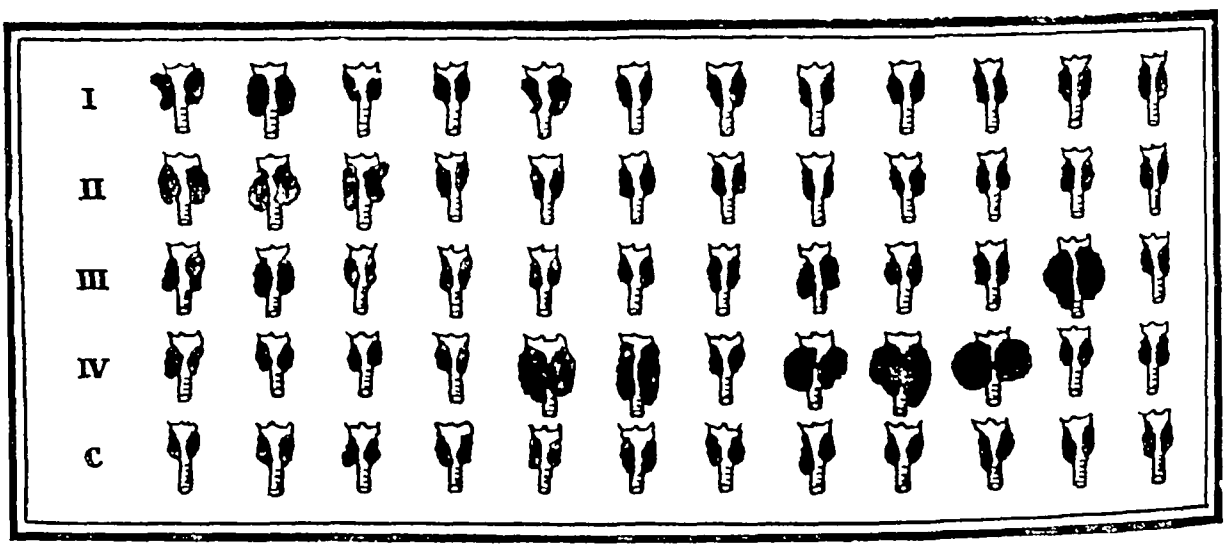
Number of animal	Sex	Original body-weight grammes	Maximum body-weight attained grammes	Final body-weight grammes	Days under experiment	Cause of death	Thyroid at post-mortem	Histological appearances of thyroids
2531	M	45	100	72	86	Asthenia	No goitre	Gland being exhausted of colloid
2532	F	42	132	129	189	Killed	Do	
2533	M	41	107	105	90	Pneumonia	Do	Colloid gland cyst
2534	F	42	108	89	123	Urinary calculus	Do	
2535	F	48	115	113	134	Do	Do	
2536	F	41	120	80	115	Do	Do	Colloid gland congestion cyst
2537	F	49	125	116	173	Do	Large goitre	Colloid gland vessels very engorged
2538	M	42	84	54	95	Enteritis	No goitre	
2539	M	42	86	72	52	Gastro-intestinal dystrophy	Slight goitre	*Early lymph-adenoid goitre*
2540	F	42	89	68	66	?	Do	Colloid gland vessels very engorged diffuse reticulo-endothelial reaction
2541	M	50	135	93	118	Urinary calculus	No goitre	Gland being exhausted of colloid
2542	M	48	139	99	118	Do	Slight goitre	*Early lymph-adenoid goitre*
			Average days under experiment =		113·2			

Table III

Giving details of the experiment in which 12 young rats were fed during the spring and summer months on diet 4, white bread, yeast, lime, iodine and water

Number of animal	Sex	Original body-weight grammes	Maximum body-weight attained grammes	Final body-weight grammes	Days under experiment	Cause of death	Thyroid at post-mortem	Histological appearances of thyroids
2543	M	47	96	61	76	Urinary calculus	No goitre	Colloid gland
2544	F	46	114	85	128	Do	Large goitre	*Lymph-adenoid goitre*
2545	M	41	62	47	55	Enteritis	No goitre	Exhausted gland
2546	F	44	120	88	172	Pyonephrosis renal calculus	Do	Colloid gland congestion
2547	F	45	110	45	174	?	Goitre	*Early lymph-adenoid goitre*
2548	F	41	98	62	117	Broncho-pneumonia	No goitre	
2549	F	50	84	56	105	Pneumonia	Do	
2550	M	42	49	43	19	Do	Do	
2551	M	41	152	142	191	Killed	Large goitre	*Lymph-adenoid goitre*
2552	F	45	122	95	186	Urinary calculus	Do	*Early lymph-adenoid goitre*
2553	F	47	126	111	191	Killed	No goitre	
2554	F	43	125	117	159	Urinary calculus	Large goitre	*Early lymph-adenoid goitre*
			Average days under experiment =		131·1			

amongst 21 males and 10 amongst 27 females, an incidence of 23 8 per cent in the former, and of 37 0 per cent in the latter The goitres were thus significantly commoner in females than in males They were classified as 'slight,' 'small' and 'large' 'slight,' when they appeared by visual examination to be less than twice the size of the normal organ, 'small,' when twice but less than four times the normal size, and 'large,' when four or more times the size of the normal organ According to this arbitrary classification there were 7 'slight,' 3 small' and 5 'large' goitres An additional indication of their size is provided by the Text-figure which represents, as nearly as possible, the sizes and shapes of the 18 thyroids in posterior view Life-size photographs are shown of two of the larger lymph-adenoid goitre (Plate XL, figs 1 and 2) in contrast with the thyroids of well-fed animals of the same body-weight



Text-figure

Free-hand drawing, posterior view, life size, of trachea with attached thyroids of 18 definitely-fed and 12 well-fed albino rats Rows I and II thyroids of 24 rats, fed on white bread, yeast, and water, amongst them are two 'small' goitres, three 'slight' goitres and one 'slight' unilateral goitre, urinary excretion of iodine by rats in these groups averaged 75 γ per litre Row III thyroids of 12 rats fed on white bread, yeast, lime and water, amongst them are one 'large' and three 'slight' goitres, urinary excretion of iodine by rats in this group averaged 85 γ per litre Row IV thyroids of 12 rats fed on white bread, yeast, lime, iodine and water, amongst them are five 'large' goitres, urinary excretion of iodine by rats in this group averaged 122 γ per litre Row C thyroids of 12 control, well-fed, albino rats, showing amongst them no goitres, urinary excretion of iodine by rats in this group averaged 70 γ per litre

The goitrous organ was often congested, its isthmus thickened and broadened, and the lateral lobes showed uniform or irregular growth in various directions lateral, lateral and downwards, downwards mainly, or lateral and posterior Occasionally, the œsophagus was almost completely enveloped by the backward growth of the lobes Quite often one lobe was larger than the other (*see* Text-figure)

A histological examination was made of 34 out of the 48 thyroids in this series—15 goitrous and 19 non-goitrous glands Their general histological appearances are given in the last column of Tables I, II and III, from which it will be seen that 9 of the 15 goitres were of lymph-adenoid type an incidence of 18 7 per cent in the 48 rats fed on the three diets The following account of the histological features of 4 of these 9 lymph-adenoid goitres was prepared at my request by Drs Williamson and Pearse who originally described this condition in man (Williamson, 1925) —

Thyroid of rat No 2544, *serial section No* 9—'In this section there are only 18 colloid follicles The epithelium of these follicles is low cubical, indeed, generally speaking, it is pressed flat and the nuclei are centrally placed (Plate XLI, fig 3) Even though post-mortem changes have rendered the epithelium in the other gland-units somewhat 'catarrhal,' the contrast between the secreting gland-units and the colloid gland-units is remarkable (the former showing high columnar epithelium and other features peculiar to active secretion) Yet another feature of the secreting follicles is the pale, washed-out, neutral cytoplasm, almost achromatic, as we first described it in lymph-adenoid goitre in the human being (Plate XLI, fig 4) But the striking features about the general body of the gland is, by contrast, the intense chromatism of the reticulo-endothelial system throughout the lobe This is transformed into a granular chromatism in the areas, widely scattered through the lobe, where lymphocytes predominate Small and large proliferated endothelial cells are aggregated together to form the typical lymphoid aggregates of lymph-adenoid goitre This picture is identical with those in your first experiment (McCarrison, 1927), which we were able to compare so completely with human lymph-adenoid goitre material' (Williamson *et al*, 1929)

Thyroid of rat No 2554, *serial section No* 9—'This specimen contrasts with the previous one in that it still contains an abundance of colloid (Plate XLI, fig 5), but there are easily recognized secretion-follicles in which our micro-capillaries (Williamson, 1923) are apparent even with your stain (hæmotoxylin and eosin) Wherever the secreting follicles occur there is an intense lymphoid activity spreading out from that central point While, generally speaking, the lymphoid aggregations are local, the reaction in the reticulo-endothelium is more general Perhaps the most important feature of this section is the widely dilated lymph-channels in which it is possible to detect the blood-vessels lying free One of the capsular lymph-spaces is packed full of cells, and many of the endothelial cells in the gland contain brown or pink, coarse granules On the whole, this specimen appears to be an earlier stage of the (lymph-adenoid) condition'

Thyroid of rat No 2551, *serial section No* 5—'This specimen closely resembles No 2554, though there are fewer colloid follicles and the lymphoid reaction is more intensely focal' (Plate XLI, fig 6)

Thyroid of rat No 2552, *serial section No* 8—'In this gland the most striking features are the very occasional lymph-aggregates (Plate XLII, fig 7)

and the very diffuse reticulo-endothelial reaction The (lymph-adenoid) process has not proceeded very far There are two follicles with well-formed walls, but from the edge of which, or from a point in the wall of which, there appears to be growing an irregular mass of epithelium This is apparently not catarrhal but actual hyperplasia of the epithelium' (Plate XLII, fig 8)

'We have seen in some of the specimens the "keratinization cysts" you describe (McCarrison, 1929) we consider them to be endothelial and not epithelial in origin'

From this description of 4 representative specimens it is evident that lymph-adenoid goitre, as originally described in the human subject by Drs Williamson and Pearse (1925), has again been produced under experimental conditions in rats by dietetic means, and that the condition is identical in man and in rats The experimental production of lymph-adenoid goitre by diets of white bread and yeast (McCarrison, 1929) is thus confirmed

Second series Autumn and winter experiments

These included (1) 12 young rats fed on diet 1 (white bread, dried yeast and water)

(2) 12 young rats fed on diet 2 (white bread, dried yeast, lime, and water)

(3) 12 young rats fed on diet 3 (white bread, dried yeast, iodine, and water)

(4) 12 young rats fed on diet 4 (white bread, dried yeast, lime, iodine and water)

The results of this series are set out in Tables IV, V, VI and VII

An auxiliary procedure was followed in evaluating the size of the thyroids in this series The usual diagnosis, as to the presence or absence of goitre and as to the size of the goitres, was made by visual examination at post-mortem (Column 10, Tables IV to VII) The glands were then removed and weighed, their weights (Column 8, Tables IV to VII) being compared with those of normal thyroids in well-fed stock rats (Column 9, Tables IV to VII)

A comparison of the results in this series with those in the first brings to light two striking differences the incidence of goitre was significantly higher, and the size of the goitres was, in general, larger in the first than in the second series Further, on histological examination, none of the 5 'slight' goitres in the second series were found to be lymph-adenoid all were colloid glands showing greater or lesser degrees of colloid exhaustion and no evidence of aggregation of lymphocytes This result is the same as that of the 1928-29 experiment (McCarrison, 1929) No case of lymph-adenoid goitre occurred in rats fed on the white-bread-and-yeast diet during the autumn and winter months

TABLE IV

Giving details of the experiment in which 12 young rats were fed during the autumn and winter months on diet 1, white bread, yeast and distilled water

Number of animal	Sex	Original body-weight grammes	Maximum body-weight attained grammes	Final body-weight grammes	Days under experiment	Cause of death	Weight of thyroid mg	Standard thyroid-weight in stock rats of same body-weight mg	Diagnosis at post-mortem and histological appearances of goitres
2597	M	50	147	146	175	Killed	9·0	12·6	No goitre
2598	F	42	122	92	113	Urinary calculus	11·0	8·7	Prominent congestion
2599	M	47	156	156	175	Killed	11·0	13·3	No goitre
2600	F	43	114	95	150	Cystitis, etc	13·0	9·0	Slight goitre colloid being exhausted no lymphocyte aggregates diffuse RE action
2601	M	49	150	150	175	Killed	8·6	12·9	No goitre
2602	F	40	119	119	175	Do	10·0	10·7	Do
2603	M	48	159	157	175	Do	7·8	13·4	Do
2604	F	50	130	130	175	Do	10·0	11·5	Do
2605	M	45	164	164	175	Do	13·6	13·9	Do
2606	F	43	109	90	106	Cystitis, etc	15·0	8·6	Slight goitre colloid gland no lymphocyte aggregates
2607	M	45	133	116	175	Killed	8·4	10·6	No goitre
2608	F	42	128	128	175	Do	11·6	11·4	Do
Averages		45·3	136	128	162		10·7		

TABLE V

Giving details of the experiment in which 12 young rats were fed during the autumn and winter months on diet 2, white bread, yeast, lime and distilled water

Number of animal	Sex	Original body-weight grammes	Maximum body-weight attained grammes	Final body-weight grammes	Days under experiment	Cause of death	Weight of thyroid mg	Standard thyroid-weight in stock rats of same body-weight mg	Diagnosis at post-mortem and histological appearances of goitres
2609	M	49	97	97	67	Urinary calculus	7·6	9·2	No goitre
2610	F.	47	107	103	175	Killed	7·2	9·6	Do
2611	M	49	97	94	175	Do	9·2	8·8	Do
2612	F	46	89	80	175	Do	6·4	7·8	Do
2613	M	42	93	93	113	Urinary calculus	7·8	8·8	Do
2614	F	48	89	87	94	Do	10·5	8·5	Prominent congestion
2615	M	50	85	70	107	Do	5·0	7·0	No goitre
2616	F.	41	79	78	75	Do	7·2	7·7	Do
2617	M	50	92	92	83	Do	7·4	8·7	Do
2618	F	41	107	104	175	Killed	8·8	9·6	Do
2619	M	41	115	110	175	Do	10·2	10·1	Do
2620	F	40	70	56	115	Urinary calculus	3·0	5·7	Do
Averages		45·3	93	88	127·4		7·5	·	

TABLE VI

Giving details of the experiment in which 12 young rats were fed during the autumn and winter months on diet 3, white bread, yeast, iodine and distilled water

Number of animal	Sex	Original body-weight grammes	Maximum body-weight attained grammes	Final body-weight grammes	Days under experiment	Cause of death	Weight of thyroid mg	Standard thyroid-weight in stock rats of same body-weight mg	Diagnosis at post-mortem and histological appearances of goitres
2621	M	41	101	88	73	Intestinal obstruction	7·4	8·5	No goitre
2622	F	48	76	61	52	Hepatic disease	13·0	6·3	*Slight to small goitre* colloid gland no lymphocyte aggregates
2623	M	43	140	140	167	Killed	14·0	12·2	No goitre
2624	F	44	123	107	112	Intestinal dystrophy	8·8	9·9	Do
2625	M	41	125	97	98	Pericarditis	10·6	9·2	Do
2626	F	46	107	67	117	Pneumonia	6·8	6·8	Do
2627	M	48	139	129	167	Killed	8·6	11·5	Do
2628	F	48	110	88	134	Urinary calculus	6·8	8·5	Do
2629	M	48	142	115	89	Pneumonia	15·0	10·5	*Very slight goitre* colloid being exhausted marked congestion no lymphocyte aggregates
2630	F	47	106	76	132	Do	8·6	7·5	No goitre
2631	M	45	92	68	101	Do	6·8	6·8	Do
2632	F	44	130	90	145	Do	5·4	8·6	Do
Averages		45·2	116	94	115·6		9·3	8·8	

TABLE VII

Giving details of the experiment in which 12 young rats were fed during the autumn and winter months on diet 4, white bread, yeast, lime, iodine and water

Number of animal	Sex	Original body-weight grammes	Maximum body-weight attained grammes	Final body-weight grammes	Days under experiment	Cause of death	Weight of thyroid mg	Standard thyroid-weight in stock rats of same body-weight mg	Diagnosis at post-mortem and histological appearances of goitres
2633	M	43	58	58	64	Urinary calculus	5·2	6·1	No goitre
2634	F	44	60	47	62	Inanition	5·0	5·0	Do
2635	M	47	73	53	77	Pneumonia	1·8	5·1	Do
2636	F	44	76	59	119	Urinary calculus	3·6	6·1	Do
2637	M	44	92	64	160	Do	5·1	6·1	Do
2638	F	49	90	63	110	?	10·1	6·3	*Slight bilateral enlargement* colloid gland no lymphocyte aggregates
2639	M	47	62	50	67	Cystitis, necrosis of kidney	5·2	5·3	No goitre
2640	F	42	86	86	119	Urinary calculus	2·6	8·1	Do
2641	M	45	53	44	145	Do	3·6	1·7	Do
2642	F	47	47	Excluded	Excluded	Pneumonia			Do
2643	M	41	64	60	87	Do	6·0	6·2	Do
2644	F	50	74	71	55	Pernicious anæmia	6·4	7·0	Do
Averages		45·2	69	59	96·8		5·3	6·1	

B Influence of season on the incidence of lymph-adenoid goitre in rats

Grouping the 1928-29 (McCarrison, 1929) and the present experiments together, the following seasonal difference in the incidence of 'goitre' is found —

	Number of animals	Number of 'goitres'	Incidence of 'goitre' per cent
Spring and summer experiments	60	19	31·6
Autumn and winter experiments	66	7	10·6

Statistically speaking, this difference is significant, it indicates that Coonoor rats, fed on the white bread and dried yeast diets, are more likely to develop 'goitre' during the spring and summer than during the autumn and winter at this altitude and in this climate

In the winter experiments of 1928-29 (McCarrison, 1929) there were only two 'goitres' (one a unilateral swelling) amongst 18 rats fed on the white-bread-and-yeast diet Neither of these was lymph-adenoid Both were colloid glands showing no evidence of lymphocytic aggregation, the unilateral swelling of the one being due, in considerable part, to a 'keratinization cyst' in the enlarged lobe In the winter experiments of 1929-30 (present series) there were 5 'slight goitres' amongst the 48 animals None of these showed any aggregation of lymphocytes, though in one (2,600, Table IV) there was a diffuse reaction of the reticulo-endothelium All were colloid glands showing greater or lesser degrees of colloid exhaustion and relatively feeble staining of the glandular epithelium

In the summer experiments of 1928 (McCarrison, 1929) there were three cases of lymph-adenoid goitre amongst 12 rats fed on the white-bread-and-yeast diet, and in the summer experiment of 1929 (present series) there were two cases of lymph-adenoid goitre amongst 24 rats fed on the same diet, or, altogether 5 cases in 36 animals While, therefore, the incidence of the condition in the winter was nil, its incidence in the summer was 13·8 per cent If the 24 rats (present series), fed during the spring and summer months of 1929 on the white-bread-and-yeast diet to which lime or lime and iodine were added, be included in the calculation then there were 12 cases of lymph-adenoid goitre amongst 60 animals (or 20 per cent) in the summer, and none amongst 66 in the winter This difference is significant, and indicates that lymph-adenoid goitre is more likely to arise, in this climate and at this altitude, in Coonoor rats when fed on the deficient diets during the spring and summer than when fed on the same deficient diets during the autumn and winter

Having regard to the effects of temperature on the thyroid gland it seems unlikely that this difference in seasonal incidence could be due to seasonal variations in atmospheric temperature *per se* Cold causes the thyroid to be depleted of colloid material and to assume the characters of 'active secretion',

heat causes colloid material to be retained in the gland In Coonoor the variations in atmospheric temperature are relatively slight During the spring and summer the maximum temperature, in the animal houses, was between 70° and 75°F , the minimum between 60° and 65°F During the autumn and winter the maximum ranged between 73° and 53°F , the minimum between 58° and 42°F It was only occasionally that the temperature fell to 50°F , very occasionally that it fell below 50°F (10°C) This degree of cold might possibly cause colloid material to be mobilized and the gland to assume the characters of 'active secretion', a result which might be expected to favour rather than to disfavour the development of a condition which, in its fully developed form, is characterized by colloid depletion The temperature conditions in Coonoor during the spring and summer months are, on the other hand, such as would favour colloid retention It would seem, therefore, that the seasonal difference in the incidence of lymph-adenoid goitre was not due to the effects on the thyroid gland of temperature *per se* Nor can it be correlated with a difference in the survival period of the animals at the two seasons Those fed on diets 1 and 2 actually lived longer, on the average, in the winter than in the summer experiments They had, therefore, more time in which to acquire lymph-adenoid goitre To all appearances the diets used during the two seasons were the same One is forced, then, to look elsewhere for an explanation of the seasonal incidence of this condition The possibility that it may be found in an 'infection' of some kind, carried by certain deficiently-fed animals and not by others and more likely to be pathogenic in summer than in winter, cannot be disregarded For although this type of goitre has not been observed to arise in well-fed animals it may be doubted whether *negative*, dietetic factors are alone concerned in its causation Its relatively low incidence in rats fed on the deficient diets is suggestive of some *positive* causal agent, while Lloyd Arnold's work on the influence of temperature and dietetic conditions in favouring 'infection' of the upper intestinal tract is pertinent in this connexion Nor can vectors of 'infection' be ignored in lice-infested rats without further inquiry While, therefore, temperature *per se* may not account for the seasonal incidence of lymph-adenoid goitre it is within the bounds of possibility that factors associated with seasonal variations in atmospheric temperature may ultimately be found to do so To me the histological features of the experimentally-produced lymph-adenoid goitres are suggestive of 'toxic' stimulation of a physiologically sub-normal organ, and at the present stage of my investigations it seems well to be alive to the possibility of the association of some *positive* agency with the *negative* one of dietetic deficiencies in causing this condition

C Influence of lime on the development of lymph-adenoid goitre in rats

Taking both summer and winter experiments together there were 48 animals fed on diets containing slaked lime, and 48 on diets not containing it Amongst

the former there were 10 goitres, amongst the latter 10, there was no difference between the two groups The addition of slaked lime to the diet of white bread and yeast had, therefore, no influence on the incidence of goitre Considering only the summer experiments, in which alone lymph-adenoid goitre occurred, and counting that of 1928 with the present series, then there were 5 lymph-adenoid goitres amongst 36 animals whose diet did not contain slaked lime, and 7 amongst 24 whose diet did contain slaked lime, a difference of doubtful significance It is notable, however, that all the large goitres occurred amongst rats to whose diet lime was added (*see* Text-figure, p 584)

Considering next the winter experiments, in which the thyroid glands were weighed (Tables IV to VII), it is found that the weight of the organ per 100 grammes of body-weight was no greater in the deficiently-fed rats whose diets contained slaked lime than in those whose diets did not contain it the average weight in the former being 8 6 mg and in the latter 9 0 mg , a result in contrast with that previously observed in well-fed animals (McCarrison, 1925) In the presence, therefore, of deficiency of fat-soluble vitamins and of phosphates an excess of lime in the diet did not cause enlargement of the thyroid gland during the winter months although the urinary excretion of calcium was very high It seems possible, however, that it may have had the effect of augmenting the size of the goitres arising, during the summer months, as a result of other faults in the experimental diets The data are, however, too meagre to admit of a definite conclusion being drawn on this point

D Influence of iodine on the development of lymph-adenoid goitre in rats

In previous reports (McCarrison, 1927, 1928, 1929 and Williamson *et al*, 1929) it has been shown that this type of goitre can arise despite the adequate ingestion of iodine,* the results of the present experiments appear to indicate that the administration of iodine to the deficiently-fed animals actually favoured its development (*see* Text-figure, p 584)

Taking the summer and winter experiments together there were 36 animals to whose deficient diet iodine was added and 60 to whose deficient diet it was not added Amongst the former there were 8 'goitres,' or 22 2 per cent, amongst the latter there were 12 'goitres,' or 20 per cent This difference is not significant But if we consider only the results of the summer experiments, in which alone lymph-adenoid goitre occurred, then there were 5 goitres of this type amongst 12 rats to whose deficient diet iodine was added, and 4 amongst 36 to whose deficient diet it was not added, an incidence of 41 6 per cent in the former and of 11 1 per cent in the latter This difference is significant Further, if the summer experiment of 1928 be included in the calculations, then there were 5 lymph-adenoid goitres amongst 12 rats to whose deficient diet

* It has been observed in rats whose urinary excretion of iodine was as high as 200,000 γ per litre

iodine was added and 7 amongst 18 to whose deficient diet it was not added, an incidence of 41 6 per cent in the former and of 14 5 per cent in the latter Again the difference is significant, it appears to indicate that lymph-adenoid goitre is more likely to arise in rats, fed on the deficient diets used in these experiments, when iodine is administered to them than when it is not This result is in accord with that of a recent survey of goitre in 2,651 rats used for experimental purposes in these laboratories during 1926-29 It was found in this survey, that the administration of iodine to deficiently-fed rats was definitely favourable to the development of 'goitre' (McCarrison, 1930*b*), while its administration to well-fed rats was not These observations suggest that different effects are produced on the thyroid gland by iodine in the presence and in the absence of a sufficiency of fat-soluble vitamins The practice is now being followed of estimating the iodine-content of one lobe of the goitrous gland while using the other for histological study One such case is shown in Plate XLII, figs 10 and 11 and Plate XLIII, fig 9 The numbers so examined are as yet few, but they indicate that the iodine-content of experimentally-produced lymph-adenoid goitres may be only 1/20th to 1/3rd of that in the normal gland even in rats to whose deficient diets iodine was added It would seem, therefore, that in the presence of certain food-deficiencies, chiefly those of fat-soluble vitamins, the capacity of the thyroid gland to deal with iodine in a normal way may be impaired, an observation which may have an important bearing on the varying results of iodine prophylaxis in different individuals In this connexion the observation of Rabinowitch (1929) is of interest, he finds that a combination of relatively minute quantities of organic iodine with a concentrated preparation of fat-soluble vitamins causes as marked a lowering of the metabolic rate in Graves' Disease as larger doses of iodine when not combined with these vitamins Fraser and Cameron (1929) have made a similar observation using small amounts of sodium iodide instead of organic iodine

E Relation of vitamin C-deficiency to the development of lymph-adenoid goitre in rats.

It has been said that deficiency of vitamin C was one of the two main faults in the diets hitherto used in the experimental production of lymph-adenoid goitre in rats One-hundred-and-one young rats were fed on synthetic diets deficient in vitamin C, but complete in other respects In none of these did goitre of any kind occur It is evident, therefore, that deficiency of this factor is not the paramount food-fault concerned in the causation of lymph-adenoid goitre in rats These animals seem to thrive fairly well on synthetic diets lacking vitamin C-containing food-stuffs, though their eagerness to secure them is, to my mind, a sufficient indication of their need for them Other animals, including man, are more sensitive to want of this factor I hesitate, therefore, to absolve vitamin C from all connexion with the development of this type of goitre, for though rats may be able to do without it man certainly cannot Further, although it may have no influence in causing lymph-adenoid

goitre in rats when it is the sole dietary defect yet its influence may not be negligible when associated with deficiency of fat-soluble vitamins It is to be remembered also that in guinea-pigs deficiency of vitamin C may cause the thyroid gland to be several times its normal size, consequent on congestion or hæmorrhagic infiltration of the organ (McCarrison, 1919), the effect on the thyroid gland of an insufficient supply of this vitamin in the food of man may not, therefore, be negligible

F Relation of vitamin D-deficiency to the development of lymph-adenoid goitre in rats

The results of the experimental study of lymph-adenoid goitre, so far as it has gone, would appear to indicate that deficiency of fat-soluble vitamins is the dominant dietetic factor on which the development of this condition depends An attempt was, therefore, made to determine which of these—A or D—was the more important Thirty-six young rats were fed on a purely vegetable diet deficient in fat-soluble vitamins and in vitamin C Eighteen of them were exposed for two hours daily, during the summer months of 1929, to the direct rays of the sun, which at this altitude and latitude are very powerful The backs and flanks of the animals were smeared with vegetable oil in order further to promote the formation of vitamin D The rats had thus opportunity to secure the activated oil from their own coats The other 18 animals were kept in the dark, their urinary excretion of iodine averaged 48 γ per litre, no goitre occurred amongst them Amongst those exposed to the sun's rays the average urinary excretion of iodine was higher (96 γ per litre), there were two cases of lymph-adenoid goitre in this group of which one is shown in Plate XLII, figs 10 and 11 and Plate XLIII, fig 9 If sunlight be a sufficient substitute for vitamin D, then the results of this experiment would appear to indicate that deficiency of this factor is not the paramount dietetic influence concerned in causing lymph-adenoid goitre in rats The experiment is, however, a preliminary one, others in which concentrates of vitamins A and D are given or withheld are now in progress, and their results will be reported at a later date

In this connexion it may be mentioned that it is the practice in these laboratories to expose all our experimental animals to the sun's rays two or three times a week As none of them have ever shown obvious signs of vitamin D-deficiency we have come to regard this practice as a sufficient substitute for the provision in the food of vitamin D-bearing substances

Summary

1 The experimental production of lymph-adenoid goitre in rats, by means of a diet composed of white bread and dried yeast, is confirmed The average urinary excretion of iodine by rats fed on this diet was 75 γ per litre

2 Rats fed on this diet are more likely to develop lymph-adenoid goitre during the spring and summer than during the autumn and winter It is considered that the seasonal incidence of the condition is not determined by the action of temperature *per se* on the thyroid gland

3 The addition of slaked lime to the diet of white bread and yeast had no influence on the incidence of the goitres caused by this diet, though it appeared to augment the size of those occurring during the summer months, its addition to the diet during the winter months did not cause an increase in size of the thyroid gland

4 The addition of iodine to this diet appeared to increase the incidence of lymph-adenoid goitre in the spring and summer, but not in the autumn and winter experiments This condition was most common in rats to whose diet iodine was added and whose urinary excretion of iodine averaged 122γ per litre

5 A description of four representative specimens of experimentally-produced lymph-adenoid goitre in rats is provided by Drs Williamson and Pearse who originally described this condition in man The experimentally-produced lymph-adenoid goitre is identical with that occurring in man

6 The faults common to the diets hitherto used in the experimental-production of lymph-adenoid goitre are (*a*) deficiency of fat-soluble vitamins (A and D), and (*b*) deficiency of vitamin C

7 Rats fed on synthetic diets deficient in vitamin C, but complete in other respects, did not develop lymph-adenoid, or other, goitre

8 Lymph-adenoid goitre occurred in 2 out of 18 rats fed on a diet having the above faults, although they were exposed daily to the direct rays of the sun, and were, presumably, acquiring a sufficiency of vitamin D in this way The average urinary excretion of iodine by rats so treated was 96γ per litre

9 By a process of exclusion of other vitamins, vitamin A-deficiency would appear to be the chief *dietetic* factor concerned in causing lymph-adenoid goitre in rats, but further experimentation is necessary before the relative importance of the fat-soluble vitamins (A and D) can be finally determined

10 It is pointed out that while vitamin-deficiency appears to be the main *dietetic* factor concerned in causing lymph-adenoid goitre in rats—without which it has not up to the present been observed to arise—the possibility has to be borne in mind that some unknown *positive* agency may be associated with the *negative* one of vitamin-deficiency in the causation of this type of goitre

11 It would appear from the results of these experiment that in the absence of a sufficiency of fat-soluble vitamins in the diet the thyroid gland is unable to deal with iodine in a normal way, and, that its administration in these circumstances may actually favour goitre-production It seems possible that the varying results of iodine-prophylaxis in different individuals may be related to the varying amounts of these vitamins in the diets

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Explanation of Plate XL

Fig 1 Showing lymph-adenoid goitre (2,547 Table III) on the right, in comparison with a normal thyroid of a female rat of the same body-weight as the goitrous animal

„ 2 Showing on the right another lymph-adenoid goitre (2,544 Table III) in comparison with a normal thyroid on the left

PLATE XL

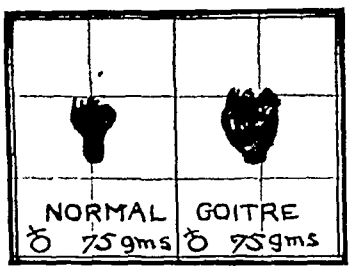

Fig 1

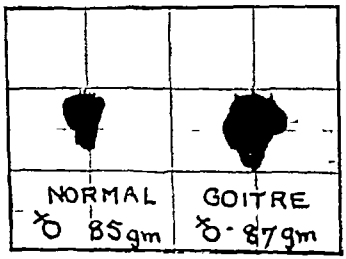

Fig 2

Explanation of Plate XLI

Figs 3 and 4 Sections of lymph-adenoid goitre (2,544 Table III), for description *see* text

Fig 5 Section of lymph-adenoid goitre (2,554 Table III) showing area of gland in which there are vesicles containing an abundance of colloid For description *see* text

„ 6 Section of lymph-adenoid goitre (2,551 Table III) showing focal lymphoid reaction

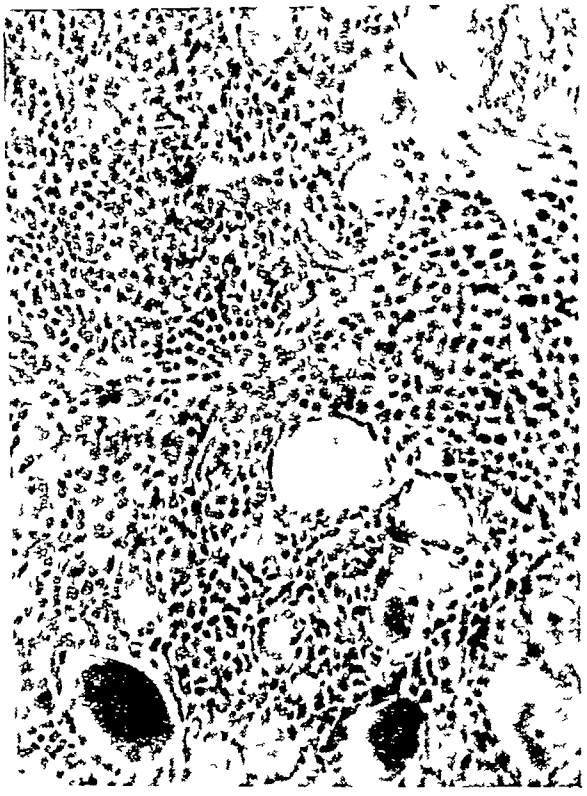

Fig 3

Fig 4

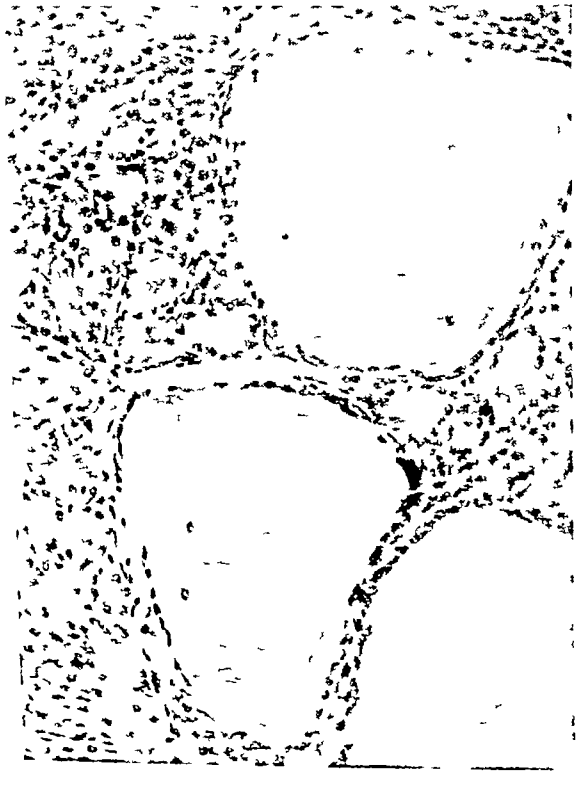

Fig 5

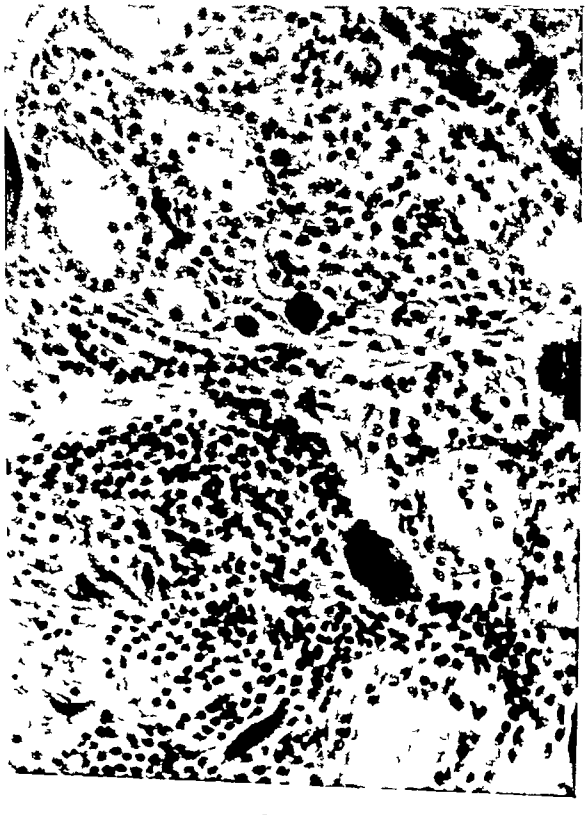

Fig 6

Explanation of Plate XLII

Fig 7 Section of lymph-adenoid goitre (2,552 Table III) showing less intense lymphoid reaction and poor chromatism of the glandular epithelium An engorged vessel occupies the central part of the figure

„ 8 Area of hyperplasia in lymph-adenoid goitre (2,552 Table III)

Figs 10 and 11 High power views of parts of Fig 9 showing the typical appearances of well-advanced lymph-adenoid goitre

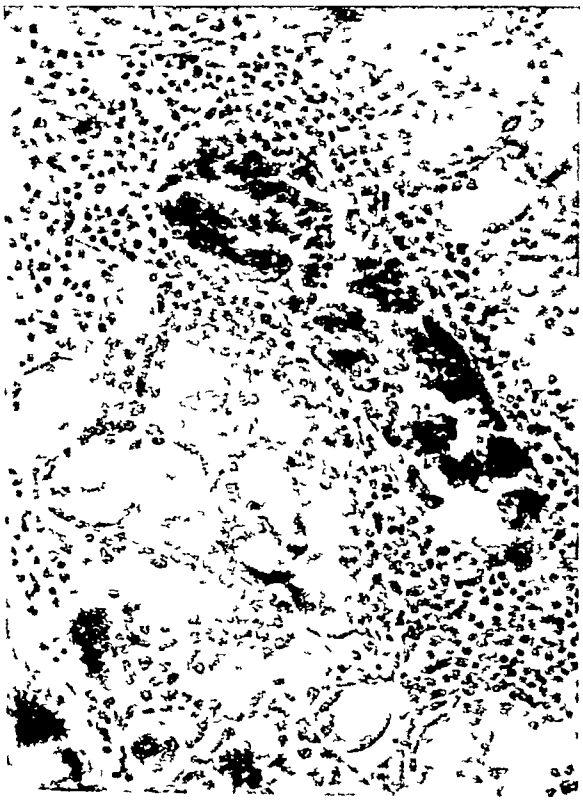

Fig 7

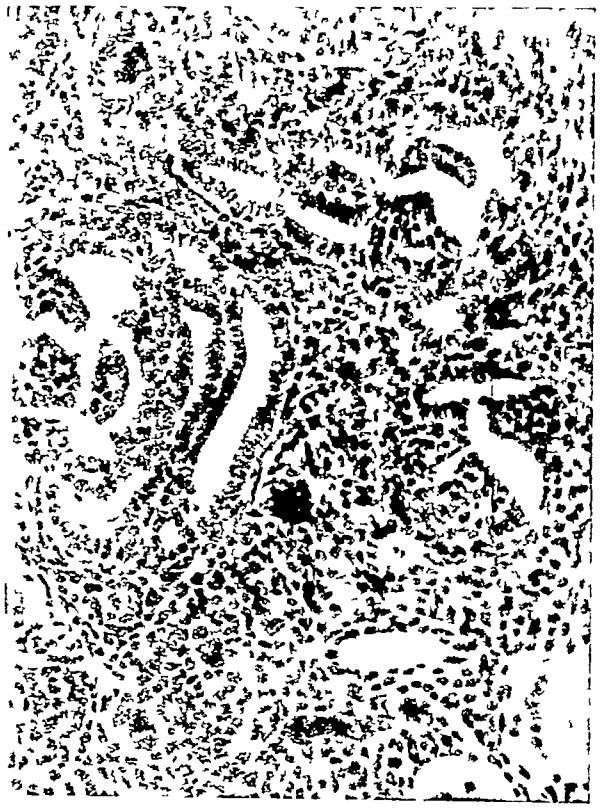

Fig 8

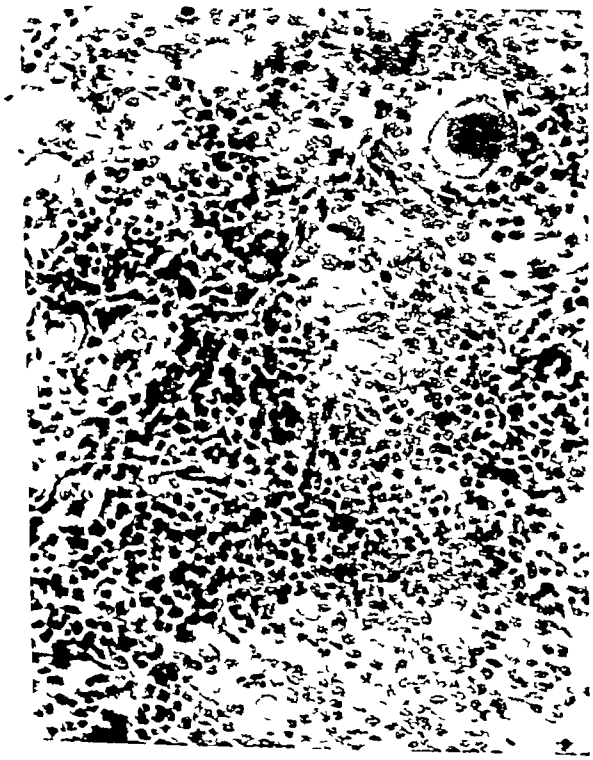

Fig 10

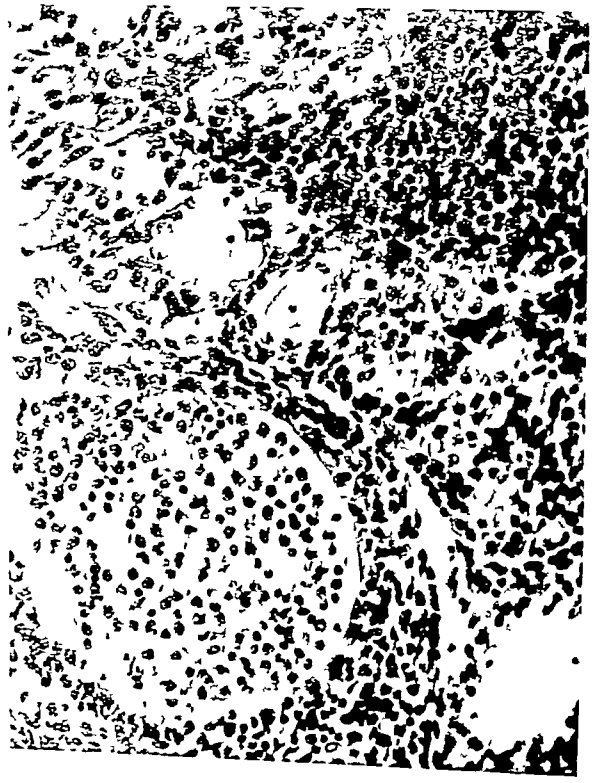

Fig 11

CHEMICAL COMPOSITION OF URINARY CALCULI IN RATS

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Material

In the course of his investigations on the causation of urinary calculus cCarrison, 1927*a*, 1927*b*, 1927*c* and 1928) Colonel McCarrison (1930) has d that by adding slaked lime to certain diets deficient in vitamin A, their producing potency is greatly increased, so much so that in certain iments there has been an almost cent per cent incidence of urinary calculi rats fed on these diets* Previous analyses of rat stones, produced by wcomb and Ranganathan, 1930), have shown them to be composed for part of ammonium magnesium phosphate, his object, for some time been to alter this composition by dietetic means A number of diets used for this purpose, and the task with which he entrusted me ke an analysis of some of the stones arising in rats fed on these diets tones were handed over to me, no particulars other than their serial ng provided until the analyses were completed, when information et was provided for purposes of correlation It was at once obvious sical characters of the stones in this series differed markedly from e previous series (Newcomb and Ranganathan, 1930) they were as e or greyish-white in colour, and usually multiple, their appearance , and often the concretions consisted of fine rounded, chalky grains, tendency to adherence into a single mass In weight they varied han 2 to as much as 800 mg Sixty were bladder stones, 15 were

* Unpublished results

(3) A rat stone, composed for the most part of calcium carbonate, was treated with normal sulphuric acid, when a flocculent, insoluble mass separated out This was centrifuged, the supernatant liquid discarded and the flocculent mass again extracted with normal sulphuric acid and treated as in (1)

All three specimens gave good titration values, but none gave a sharp and definite end-point It was concluded, therefore, that the titration values obtained in rat stones were due to the presence of organic matter and not to the presence of oxalates, a conclusion confirmed by the failure to detect oxalic acid in these stones by qualitative tests Hence it was that in stones which did not permit of a complete, quantitative, micro-chemical analysis, estimation of oxalates was not done, even when the estimation was done in larger stones, the titration values were discarded, because the values obtained in most instances were far higher than could theoretically be expected, and because of the lack of confirmatory evidence by the qualitative tests

(c) *Qualitative analysis of stones weighing* 5 *milligrams or less*—Of such stones there were 25 in the present series of 78 Each stone was powdered and the powder divided into three approximately equal portions, two being transferred to two micro-test-tubes (0 5 × 5 0 cms , capacity about 1 5 c c and usually weighing about a gramme) and the third to a watch-glass

To the first tube three drops of concentrated nitric acid were added, effervescence, indicating the probable presence of carbonates, commonly occurred Three drops of phosphate reagent (ammonium molybdate in concentrated nitric acid) was then added and the tube slightly warmed, when if phosphates were present there appeared the characteristic, golden-yellow precipitate The limit of detectability of phosphates by this test was found, in a series of controls, to be as low as 0 01 of a milligram of P_2O_5

To the second tube three drops of normal sulphuric acid were added, the presence of carbonates being indicated by a brisk effervescence A tiny drop of methyl red, followed by two drops of a saturated solution of ammonium oxalate, was then added and the contents neutralized to methyl red by ammonia The tube was warmed in a water-bath, a precipitate indicated the presence of calcium, an indication confirmed by the insolubility of the precipitate in acetic acid If calcium was not present or if present only in traces, as indicated by a slight opalescence, then two drops of a strong solution of sodium phosphate, followed by two drops of concentrated ammonia, were added The tube was violently shaken and examined after 10 minutes A crystalline precipitate indicated the presence of magnesium, magnesium was tested for only in the absence, or doubtful presence, of calcium

The third fraction in the watch-glass was mixed with 2 or 3 drops of concentrated nitric acid and evaporated to dryness on a slow electric heater After cooling, a drop or two of very dilute ammonium hydroxide was added when, if uric acid was present, a deep purple colour appeared One-fiftieth of a milligram of uric acid can be detected in this way

The results of these analyses are set out in Table I

TABLE I

Showing the chemical composition of 25 rat stones that weighed 5 milligrams or less

Directions + (?) = Doubtful or faint trace, + = Present, ++ = Fair amounts, +++ = Plenty, 0 = Nil

Stone number	Carbonate	Phosphate	Calcium	Magnesium	Murexide test	Diet	Location of stone
24	+++	+ (?)	+++		0	A	Bladder
25	++	++	+ (?)	++	0	D	Do
29	+	+ (?)	+++		0	C	Do
32	+++	0	+++		0	B	Kidney
38	+++	+	+++		0	B	Do
39	+	+ (?)	++		0	H	Bladder
40	+	0	+++		0	A	Do
44	+++	+ (?)	+++		0	C	Do
47	+++	0	+++		0	B	Do
49	+ (?)	+ (?)	+++		0	C	Kidney
57	+ (?)	+ (?)	0	++	0	D	Bladder
60	+++	+	+++		0	A	Do
62	+++	+ (?)	++		0	C	Kidney
64	+++	+	++		0	E	Do
65	+ (?)	++	+ (?)	+	0	F	Bladder
68	+++	+ (?)	++		0	E	Kidney
69	+	++	+ (?)	++	0	G	Bladder
70	+++	+ (?)	+++		0	C	Do
73	+++	+ (?)	+++		0	E	Do
74	+++	0	+++		0	E	Kidney
80	+++	+	+++		0	C	Do
81	+	0	+++		0	C	Do
85	+	0	+++		0	E	Do
105	+	0	+++		0	K	Bladder
107	0	+++	0	+++	0	L	Do

(*d*) *Quantitative analysis of stones weighing 5 milligrams or more*—Of such stones there were 53 The micro-chemical methods adopted in the

analysis were those described in a previous paper (Newcomb, 1930) The results of their analysis are set out in Table II

TABLE II

Showing the chemical composition of 53 rat stones weighing 5 mg or more

Stone number	Weight of stone mg	Moisture Per cent	As percentages on moisture-free sample					Murexide test	Diet	Location of stone
			Total nitrogen	P_2O_5	CaO	MgO	CO_2			
23	30·3	5·5	2·4	Trace	43·0	2·0	43·4	0	A	Kidney
26	127·6	27·4	0·7	0·0	29·0	0·0	0·0	0	A	Bladder
27	26·1	3·8	1·8	1·6	42·0	2·0	37·0	0	B	Do
31	117·8	36·7	1·1	0·0	35·6	0·0	0·0	0	B	Do
33	96·0	19·4	1·1	1·4	45·5	2·0	39·5	0	A	Do
34	17·3	8·7	1·2	Trace	29·0	1·8	22·0	0	C	Do
35	69·3	5·8	1·3	1·1	44·4	1·5	37·1	0	C	Glans penis
36	12·3	6·5	3·4	1·9	33·4	3·6	41·1	0	A	Bladder
37	797·8	3·5	0·5	1·1	47·6	1·6	36·0	0	B	Do
42	104·2	11·4	0·7	0·0	41·5	0·0	7·0	0	B	Do
43	99·8	43·8	1·1	Trace	28·0	0·0	Trace	0	A	Do
45	81·2	6·0	1·2	0·9	45·0	1·8	35·6	0	C	Do
46	43·5	10·1	1·6	Trace	30·7	Trace	21·5	0	A	Do
48	20·6	11·6	1·4	0·0	32·4	0·0	0·0	0	C	Do
50	30·7	8·5	1·5	Trace	37·0	1·4	25·5	0	E	Do
51	18·5	11·4	2·4	0·0	35·5	0·0	0·0	0	C	Do
52	59·3	5·1	1·1	0·8	42·6	Trace	29·1	0	C	Do
53	286·3	13·3	0·5	0·9	40·3	2·0	26·5	0	E	Do
54	37·5	18·4	1·1	2·7	43·2	1·6	38·4	0	E	Do
55	43·9	5·0	1·5	1·3	40·0	Trace	22·2	0	C	Do
56	245·4	19·0	0·9	0·7	29·9	0·0	8·6	0	B	Do
59	370·0	35·5	0·7	0·3	29·1	Trace	5·1	0	A	Do
61	13·7	8·8	2·3	1·0	38·1	0·0	22·2	0	C	Do
63	59·2	11·6	1·2	0·3	45·7	0·0	5·4	0	C	Do
66	200·9	19·9	1·5	0·7	32·6	3·5	12·3	0	E	Do
67	21·6	7·9	3·6	1·8	32·4	2·5	28·8	0	E	Kidney

TABLE II—*concld*

Stone number	Weight of stone mg	Moisture Per cent	AS PERCENTAGES ON MOISTURE-FREE SAMPLE					Murexide test	Diet	Location of stone
			Total nitrogen	P_2O_5	CaO	MgO	CO_2			
71	84·2	9·2	0·8	Trace	11·5	0·0	10·6	0	C	Bladder
72	20·6	8·7	3·2	0·9	11·5	0·0	Trace	0	C	Kidney
75	32·5	3·7	0·6	1·3	11·2	1·0	37·5	0	A	Bladder
76	51·3	7·8	1·8	1·2	38·0	1·6	33·7	0	B	Do
77	288·6	8·6	0·5	0·5	13·7	1·1	11·8	0	B	Do
78	72·9	11·3	0·9	0·5	37·3	1·0	21·5	0	C	Do
79	15·9	5·7	3·5	1·3	39·7	3·0	39·7	0	C	Ureters
82	112·7	29·7	0·7	0·5	27·8	0·0	Trace	0	H	Bladder
83	123·0	18·1	0·6	0·1	29·5	0·0	Trace	0	I	Do
84	73·0	9·1	1·6	0·3	10·8	0·0	7·5	0	A	Do
86	122·1	18·0	1·5	0·9	13·7	1·2	36·2	0	E	Kidney
87	83·6	22·1	0·9	0·5	11·6	2·2	19·9	0	E	Bladder
88	18·9	7·1	3·5	1·8	31·6	3·2	23·3	0	C	Kidney
89	14·6	11·0	2·9	1·5	31·0	1·8	28·6	0	C	Bladder
91	550·7	19·3	1·1	0·3	29·4	0·0	Trace	0	A	Do
92	17·5	4·6	1·7	1·9	41·1	1·5	39·1	0	A	Do
93	36·7	19·7	0·7	0·7	53·5	Trace	34·9	0	A	Do
94	51·7	3·5	0·1	1·6	43·2	2·1	39·0	0	E	Do
95	237·2	41·9	1·5	0·7	26·7	0·0	Trace	0	B	Do
96	59·5	2·7	1·1	1·4	27·6	0·0	9·6	0	B	Do
98	13·4	12·0	2·1	11·6	37·0	2·1	Trace	0	E	Do
99	13·1	3·8	1·0	2·3	44·7	3·2	42·3	0	E	Do
101	163·5	2·6	0·2	1·2	48·0	1·6	39·9	0	B	Do
103	11·9	9·3	1·9	2·2	35·3	1·2	39·0	0	K	Do
104	6·3	4·8	2·5	1·9	41·6	2·8	++*	0	A	Ureters
106	70·2	3·9	0·5	1·7	44·6	2·2	43·8	0	K	Bladder
108	11·0	39·1	5·0	36·8	4·3	24·4	Trace*	0	M	Do

*Tested only qualitatively as there was not enough stone-material for quantitative work

Results of the analyses

It will be seen from Table I, that of the 25 stones weighing less than 5 milligrams, only 5 contained appreciable amounts of magnesium and phosphates, with traces of calcium The remaining 20 stones were rich in calcium with little or no phosphates in them Most of such stones were also rich in carbonates, while there were a few which contained carbonates only in small amounts or in traces It will be seen from Table II that stone No 108 differs from the rest in being very rich in phosphates and magnesium, comparatively rich in nitrogen, and very poor in calcium and carbonates Because of insufficiency of material, ammonia was not tested for in the 6 stones in this series which were found to be rich in magnesium and phosphates, it is, however, assumed that the magnesium existed as the ammonium magnesium phosphate This assumption is warranted by the positive tests for ammonia given by some stones, reported previously (Newcomb and Ranganathan, 1930), wherein there was a sufficiency of material for the performance of this test

Omitting stone No 108 from the following consideration of the results of the quantitative analyses, it will be seen from Table II that nitrogen occurred only in small amounts, varying from 0 2 to 3 5 per cent, the average being 1 43 per cent The Murexide test showed that it did not exist as uric acid, the nature of the nitrogenous compound could not, however, be determined as it occurred in such small amounts and there was not a sufficiency of stone-material to admit of its determination Phosphates, too, occurred only in traces, the average content being 1 12 per cent The stones were rich in calcium and carbonates, the average content being 38 16 and 21 8 per cent respectively of the dry weight of the stones Magnesium was present only in small amounts, the average being 1 16 per cent

It will also be seen from Tables I and II that while there are some stones containing fairly large amounts of calcium, with approximately equivalent amounts of carbonate, there are some equally rich in calcium with little or no carbonates Qualitative tests for the presence of sulphates, cystine, formate, acetate, butyrate, oxalate and succinate showed that the calcium is not held in combination with any of these radicals, it could not exist in combination with any organic nitrogenous compound, commonly found in urine, since the stones contain nitrogen only in traces It was, therefore, suspected that the calcium might exist as its hydroxide Such stones (containing fair amounts of calcium with little or no carbonates) when treated with a small quantity of distilled water were alkaline to litmus and methyl red But the addition of a drop of 0 1 N sulphuric acid gave a distinctly acid reaction, which again turned alkaline after violent shaking and gentle warming The following experiment repeated with six stones (Nos 48, 53, 59, 66, 91 and 95) was, therefore, designed to prove that stones containing fair amounts of calcium with little or no carbonates are composed for the most part of calcium hydroxide Weighed amounts of the dry, powdered stones were treated with an excess of decinormal sulphuric acid and boiled for over five minutes, when

cold they were back-titrated against decinormal alkali with a drop of methyl red as indicator In all 6 stones, the acid used up was appreciable, but the amounts used up were not strictly equivalent to the amount of calcium present in the stones Presumably, the molecules of calcium hydroxide are coated with a thin film of some urinary colloid which renders the penetration of acid difficult, and at best, imperfect This may possibly explain why such stone-powders did not use up appreciable amounts of acid when titrated direct However, the same stone-powders, when boiled with excess of acid, took up very appreciable amounts of it Even then, the process of neutralization was imperfect, for the residue left over after the end-point was reached, when freed completely from acid by repeated washings with distilled water, and boiled as before with decinormal sulphuric acid, was found to use up a portion of the acid added Further, the stones, containing large amounts of calcium with little or no carbonates, liberated ammonia when boiled with a solution of ammonium chloride These tests show that the calcium in such stones exists as calcium hydroxide

The moisture-content of the stones varied widely from 2 1 to 49 3 per cent the average being 14 17 per cent Comparing the moisture-content of the stones with their general composition (Table III), it is seen that the moisture-content serves as an approximate guide to the composition of the stone those with a low moisture-content, below 10 per cent, and especially those below 5 per cent, consist mostly of calcium carbonate, those rich in moisture, viz, above 20 per cent, consist for the most part of calcium hydroxide, and those whose moisture-content lies between 10 and 20 per cent, consist mostly of mixtures of calcium carbonate and calcium hydroxide in varying proportions

TABLE III

Showing the influence of moisture on the chemical composition

Moisture-content	Number of calcium carbonate stones	Number of calcium hydroxide stones	Number of mixed stones	Total
5 0 per cent and below	10	0	1	11
Between 5·0 and 10·0 per cent	14	1	2	17
Between 10·0 and 20 0 per cent	5	4	7	16
Over 20 0 per cent	0	6	2	8
TOTALS	29	11	12	52

Composition of stones from various parts of the urinary tract

There were in the present series of 78 stones 60 bladder stones, 15 kidney stones, 2 ureter stones and one chalky gravel from the prepuce There was not

in this series any particular association between the location of the stone and its composition Data relating to the location of the stone and chemical composition are shown in Table IV, in the construction of which the principal constituent of the stones is reckoned

TABLE IV

Showing the location and the general composition of stones

Location of stones	Number of stones	Number of calcium carbonate stones	Number of calcium hydroxide stones	Number of mixed stones	Number of magnesium ammonium phosphate stones	Total
Bladder	60	27	11	16	6	60
Kidney	15	10	2	3	0	15
Ureters	2	2	0	0	0	2
Prepuce	1	1	0	0	0	1
TOTALS	78	40	13	19	6	78

Diet and chemical composition

Of the 78 stones, whose chemical composition was investigated, 72 contained large amounts of calcium with very little phosphate, while 6 contained appreciable amounts of phosphates and magnesium with traces of calcium The whole set of stones range themselves into two distinct groups calcium stones, wherein the calcium exists either as the carbonate or the hydroxide or a mixture of both, and magnesium ammonium phosphate stones

Tables V*a* and V*b* show the diets employed by Colonel McCarrison in the production of the present series of 78 stones

TABLE V*a*

Showing the composition of deficient diets containing added lime

A	White bread	97 per cent
	Dried yeast	3 „ „
	Lime	5 grains per rat per day
	Iodine solution (1 mg of iodine per litre)	5 drops per rat per day

TABLE Va—*concld*

B White bread	97 per cent
Dried yeast	3 „ „
Lime	5 grams per rat per day
C White bread	95 per cent
Dried yeast	3 „ „
Gingelli oil	2 „ „
Lime	5 grams per rat per day
Iodine solution (1 mg of iodine per litre)	5 drops per rat per day
E White bread	97 per cent
Dried yeast	3 „ „
Lime	5 grams per rat per day
Iodine solution (1 mg of iodine per litre)	5 drops per rat per day
Manganese chloride	0·0327 mg per rat per day
H White bread	97 per cent
Dried yeast	3 „ „
Lime	5 grams per rat per day
Distilled water containing 0·25 mg of iodine per litre of water for drinking purposes	
I White bread	95 per cent
Dried yeast	3 „ „
Radiostoleum in gingelli oil	2 „ „
Iodine solution (1 mg of iodine per litre)	5 drops per rat per day
Lime	5 grams per rat per day
J White bread	97 per cent
Dried yeast	3 „ „
Iodine solution (1 mg of iodine per litre)	5 drops per rat per day
Lime	2½ grams per rat per day

TABLE Vb

Showing the composition of deficient diets containing no added lime

D	Oatmeal	53 per cent
	Linseed meal	20 „ „
	Corn flour	25 „ „
	Sodium chloride	1 „ „
	Calcium phosphate	1 „ „
F	White flour	90 „ „
	Linseed oil	8 „ „
	Sodium chloride	1 „ „
	Calcium phosphate	1 „ „
G	White flour	20 „ „
	Casein	60 „ „
	Olive oil	8 „ „
	Gingelli oil	2 „ „
	Salt mixture (without potassium iodide)	5 „ „
	Dried yeast	5 „ „
K	White flour	60 „ „
	Casein	20 „ „
	Hydrogenated fat	8 „ „
	Hydrogenated fat plus radiostoleum	2 „ „
	Salt mixture (without potassium iodide)	5 „ „
	Dried yeast	5 „ „
L	Casein	80 „ „
	Hydrogenated fat	8 „ „
	Hydrogenated fat plus radiostoleum	2 „ „
	Salt mixture (without potassium iodide)	5 „ „
	Dried yeast	5 „ „

A study of Tables I, II, Va and Vb reveals a close association between the diet and the chemical composition of the stones This association is clearly set forth in Table VI, in the construction of which only the principal constituent of the stones is taken into consideration, Table VII shows a similar

association after incorporating the results of the previous report (Newcomb and Ranganathan, 1930)

TABLE VI

Showing the association between diet and chemical composition

Diet	Number of stones	Number of calcium carbonate stones	Number of calcium hydroxide stones	Number of magnesium ammonium phosphate stones	Number of mixed calcium stones	Total
A	16	9	3	0	4	16
B	13	8	2	0	3	13
C	22	9	4	0	9	22
D	2	0	0	2	0	2
E	15	8	1	0	6	15
F	1	0	0	1	0	1
H	1	0	0	1	0	1
I	2	0	1	0	1	2
J	1	0	1	0	0	1
G	3	2	0	0	1	3
K	1	0	0	1	0	1
L	1	0	0	1	0	1
TOTALS	78	36	12	6	24	78

TABLE VII

Showing the association between diet and chemical composition

[This includes results of the previous report (Newcomb and Ranganathan, 1930) also]

Diet	Number of stones	Number of calcium stones	Number of magnesium ammonium phosphate stones
Those containing *added lime*	73	73	0
Those containing *no added lime*	27	0	27
TOTAL	100	73	27

It is thus seen that the stones, produced on diets to which extra lime was added, were composed either of calcium carbonate or calcium hydroxide or a

mixture of both, while the stones produced on diets to which lime was not added were composed of magnesium ammonium phosphate The same association was observed in the previous series of 22 stones (Newcomb and Ranganathan, 1930) It is also seen that there is no association between the diet and the particular kind of calcium stones produced, for, the same diets that produced calcium carbonate stones in some rats produced calcium hydroxide or mixed stones in others Possibly, the stones originate as calcium hydroxide and get deposited as such, in the absence of any available carbonate in the urine Further, it is evident from the foregoing observations that the addition of lime to the diet materially alters the composition of the stone While it is generally contended that very little calcium passes through the urinary tract, and that most of the calcium ingested is excreted in the bowels, Colonel McCarrison has found calcium in the urinary tract of one rat in as large amounts as a thirtieth of the body-weight of the animal Particular interest therefore attaches to the chemical composition of the present series of stones, as it affords material for evaluating the influence of added lime on the composition of rat stones The biochemical tracing of the course of the ingested lime is being investigated and the results will be communicated later

Summary and conclusions

(1) The chemical composition of 78 urinary stones, produced by Colonel McCarrison in albino rats by means of various deficient diets, is reported

(2) There seems to be a close relation between the diet and the chemical composition of the stones, stones rich in calcium were produced on diets to which extra lime was added, while magnesium ammonium phosphate stones were produced on diets to which lime was not added

(3) Stones rich in calcium contain this element either as the carbonate or the hydroxide or a mixture of both

(4) Stones rich in calcium were comparatively poor in phosphates, there was little nitrogen in them and no uric acid Even the magnesium ammonium phosphate stones, though containing fair amounts of nitrogen, did not contain any uric acid

(5) The moisture-content of the stones varied widely from 2 1 to 49 3 per cent It serves as an approximate guide to the general composition of the stones, those relatively poor in moisture, were composed of calcium carbonate, those rich in moisture were composed of calcium hydroxide and those moderately rich in moisture were composed of a mixture of calcium carbonate and calcium hydroxide

(6) There was in this series of 78 stones no association between the location of a stone and its chemical composition

(7) The influence of the ingested lime on the urinary excretion of calcium is briefly indicated

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Idem (1928)	*Ibid*,	3,	p 801
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Newcomb (1930)	*Ibid*,	3,	p 735
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CHEMICAL COMPOSITION OF THE 'NUCLEUS' OF URINARY CALCULI

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Introduction

STATEMENTS in the literature regarding the composition of the nuclei of urinary calculi* are conflicting Thus, Langen (1929) states that 'nuclei are built up of calcium, phosphates and magnesium Besides organic ingredients are regularly found in them, sometimes still recognizable leucocytes and chemically traces of blood In the nucleus of the stone we found only now and again small quantities of oxalates and of urates, in the outer layers of the stone, however, these substances are often found in great quantities and they form the chief ingredient with respect to structure and consistency of the fully developed bladder stones Especially the composition of the nuclei is very much the same in both kinds (i e, stones from human beings and those obtained by animal experiments), while the layers enveloping the nucleus, which have developed afterwards, show more differences'

Iwano (1923) believes that the composition of 'the nucleus is of great importance with regard to its (the stone's) formation' and that it (the nucleus) has distinct relation to the age of the patient 'Uric acid and urate nuclei appear,' he says, 'much more at advanced age, while phosphate nuclei occur most frequently in persons of ages from 30 to 60 years and far less in young persons Oxalate nuclei are formed most frequently in persons between the first and fifth decennium' Gideon Wells (1925) considers that in all pathological concretions, including urinary calculi, 'there must first be a *nucleus* of some substance different from the substance that is to be deposited, and which is most frequently a mass of desquamated cells but may consist of clumped bacteria, masses of mucus, precipitated proteins or a foreign body of almost

* Vesical calculi, as is well known, may form around foreign material introduced artificially in the bladder The present investigation deals with the nuclei of stones arising without this adventitious aid

any sort' Contrary to the above, Hampton Young (1927) observes that the nucleus is usually a solidified agglomeration of the same salts of which the calculi are composed and forms the core Lastly, Shattock (1911), after an exhaustive study of the microscopic structure of urinary calculi, has shown that a nucleus of cells or other organic material is, at least in uric acid calculi, extremely rare, the centre being almost always a primary crystalline deposit from a supersaturated solution

The present investigation was undertaken to ascertain how far these statements applied to the nuclei of the stones in Colonel McCarrison's collection

Chemical composition.

Considerable difficulty was experienced in finding the nucleus first, because it did not always exist in the centre of the stone, though a centrally situated nucleus with several concentric layers around it is a common arrangement, second, because of the varying size of the nuclei which may range from a tiny point to a fairly large mass weighing as much as 70 milligrams, third, because of the existence of more than one nucleus in the same stone, and fourth, because of the absence of a nucleus in some stones

There were 61 whole stones and 189 crushed ones in Colonel McCarrison's collection A cross-section passing through the geometric centre was made in

TABLE I

Showing the chemical composition of nuclei

Nucleus of stone number	Moisture Per cent	As percentages on moisture-free sample						CO_2	Murexide test	Ammonia	Weight of nucleus in mg
		Total nitrogen	P_2O_5	CaO	MgO	C_2O_3	Insoluble ash				
8	4 1	3 6	1 6	14 6	0	22 7	32 0	0	++	—	14 5
20	3 1	9·0	0 8	28 8	0	47 0	0 0	0	++	—	16 0
25	1 1	32·7	Trace	6 3	0	7 7	0 0	0	++	—	28 4
28	0·7	34 2	Trace	1 3	0	1 2	0 0	0	++	+	40 8
29	0 0	34 5	Trace	1 6	0	3 2	0 0	0	++	+	45 5
46	1 1	34 0	Trace	Trace	0	2·8	0 0	0	++	+	37·8
61	3·2	1 6	0 9	40 5	0	43 2	0 0	0	+	0	18·6
95	0·3	33 5	Trace	4 4	0	5 3	0 0	0	++	+ (?)	39 9
139	1 2	21 7	0 8	15 0	0	17 8	0 0	0	++	+	50·4
150	2 8	34 0	0 9	4 4	0	7·4	0 0	0	++	+	14 2
163	4 0	33 5	1 8	10 4	0		0 0	0	++	+	9 9
187	0 9	0·5	4 1	39 7	0	41 6	0 0	0	+ (?)	0	7·7

the whole stones The cut surface was cleaned and examined under a hand magnifying lens Only twelve stones were found to have prominent nuclei large enough to admit of a micro-chemical analysis, in the remaining 49 whole stones, the nuclei were either indistinct or not bigger than a tiny speck The prominent nuclei were scooped out carefully and a complete quantitative chemical analysis made on them by micro-methods, moisture, ash insoluble in sulphuric acid, total nitrogen, phosphate, calcium, magnesium and oxalates were thereby determined Qualitative tests for the presence of uric acid (Murexide test), ammonia and carbonates were also done on them The results of the chemical analysis are shown in Table I

Table II shows the chemical composition of the stones from which the nuclei were separated

TABLE II

Showing the chemical composition of the stones from which the nuclei were removed

Stone number	Moisture Per cent	As percentages on moisture-free sample								CO_2	Ammonia	Murexide test	Wt of stone in gs
		Ash	Insoluble ash	Soluble ash	Total nitrogen	P_2O_5	CaO	MgO	C_2O_3				
8	14 4	63·5	34 8	28·7	4 0	10·2	12·3	5 9	13 6	0	0	+	11 0
20	9 3	37·2	Trace	37 2	14 2	11 0	20·4	Trace	16·7	0	0	++	34 0
25	1 5	2·8	0·0	2·8	30·3	0·0	2 5	Trace	3 0	0	0	++	25 0
28	1 4	0 2	0·0	0·2	32·9	0·0	0·2	0·0	0 0	0	0	++	11 0
29	3·6	3 7	0·0	3 7	31 3	1 6	0·7	0·7	0 0	0	0	++	30·0
46	1 2	1 1	0·0	1 1	32·6	Trace	0·3	0 0	0 0	0	0	++	10·6
61	3 4	38·0	0·0	38·0	0·7	Trace	36·0	Trace	44 5	0	0	+ (?)	40·0
95	1 9	1 0	0·0	1 0	32·9	Trace	0·9	0·0	Trace	0	0	++	27 0
139	9·6	18 6	0·0	18·6	23 8	6 9	7 9	3 7	7 5	0	0	++	66 0
150	36 4	63·0	Trace	63 0	11 2	37 6	1 8	22·6	0·0	0	0	++	11 2
163	2·7	27 8	0·0	27·8	12·8	3·9	22·4	0·0	24 6	0	0	++	41 0
187	2·2		0·0		1 4	9 4	33·7	2·6	26·4	0	0	+	14 0

(The stones were analysed by macro-methods)

It will be seen from Table I that the nuclei, though appearing homogeneous under the magnifying lens, were not composed of any one single substance Phosphates occurred only in traces, while urates and uric acid, and oxalates formed the chief constituents of the nuclei Seven nuclei contained uric acid

and urate for the most part, four contained calcium oxalate, and only one contained a mixture of uric acid or urate and calcium oxalate The percentage of nitrogen was in some nuclei slightly higher than it should have been had it existed solely as uric acid, presumably because of the presence of ammonium urate The presence of ammonia in such nuclei was confirmed by the qualitative test

From a study of Tables I and II it is apparent that,

(1) the chemical composition of the nuclei did not always run parallel with that of the stones enveloping them, though an approximate parallelism existed in a good many instances,

(2) the nuclei were exceptionally poor in moisture, the percentage ranging from 0 to 1 1, with an average of 1 9 per cent, of the weight of the nuclei,

(3) insoluble ash occurred only in one instance (No 8) where the stone and its nucleus contained it in nearly the same amounts,

(4) phosphates occurred only in traces in the nuclei, the average content being 0 9 per cent, the stones enclosing them contained it in much larger amounts, the average being 6 7 per cent of the weight of the moisture-free stone

Age of patient and chemical composition of the nucleus

Table III shows the ages of the patients from whom the stones were removed and the chief constituent of the nuclei

TABLE III

Nucleus of stone number	Age in years	Sex	Chief constituent of nucleus
8	7	M	Calcium oxalate
20	13	M	Do
25	61	M	Uric acid or urate
28	40	F	Do
29	50	F	Do
46	4	M	Do
61	30	M	Calcium oxalate
95	30	M	Uric acid or urate
139	12	M	Uric acid or urate and calcium oxalate
150	3	M	Uric acid or urate
163	55	M	Do
187	10	M	Calcium oxalate

It will be seen from the above table that uric acid nuclei occurred as well in the very young (3 and 4 years old) as in persons of advanced age (50, 55 and 61 years) So far as these observations go there was no relationship between the age of the subject and the chemical composition of the nuclei The observations do not support the conclusion of Iwano (1923)

Discussion.

Langen (1929) has stated that the nuclei of bladder stones are made up of calcium, phosphates and magnesium with occasional traces of oxalates and of urates, and that the nuclei of human stones and of stones experimentally produced in rats have the same composition It may be assumed, therefore, that uric acid or urate was occasionally found in traces by Langen in the nuclei of rat stones This has not been our experience, for, in an analysis made by the author of 101 urinary calculi produced experimentally by Colonel McCarrison in albino rats, uric acid was not even once detected The limit of detectability of uric acid by the Murexide test is less than a fiftieth of a milligram and hence there is no possibility of missing uric acid even if it had existed in traces Further, most of the stones, experimentally produced in rats in this laboratory were very small—about 25 per cent of them were of a size which did not admit even of a micro-chemical analysis—consequently the difficulties of getting at the nuclei and of making a chemical analysis of them are so great that a definite pronouncement as to their chemical composition is not, in my opinion, possible

Summary and conclusions

1 The chemical composition of the nuclei of 12 vesical calculi is reported

2 The nuclei, though appearing homogenous under the magnifying lens, were not composed of any one single constituent

3 Uric acid or urates formed the chief constituents of the nuclei, phosphates occurred only in traces

4 The composition of the nuclei did not always run parallel with that of the stones containing them, though an approximate parallelism existed in a good many instances

5 There seems from this limited series of observations to be no relationship between the age of the subject and the chemical composition of the nuclei of the stone

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THE EFFECTS OF HIGH PROTEIN DIETS ON THE THYROID GLAND

Part I

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Introduction.

IN 1904 C Watson reported the production of thyroid hyperplasia in fowls following the prolonged administration of raw meat He made a similar observation in rats in 1906, and in 1907, he found that wild rats kept in captivity, and fed on bread and milk, developed thyroid hyperplasia Marine (1914) observed thyroid hyperplasia in brook trout fed on a diet of liver and heart-muscle, a change of diet to fresh sea-fish was associated with an involution of this condition, the supposition being that the hyperplasia was related to a low iodine-content of the former, and its involution to a high iodine-content of the latter, diet The same observer (1915) noted the occurrence of thyroid hypertrophy in young rats when fed on liver which was one, two or three days old, but not in those fed on fresh liver or on liver which was five or six days old In 1917, Burget reported the results of his 'Attempts to produce experimental thyroid hyperplasia' He found that 'adult rats kept under hygienic conditions and fed on a high protein diet develop hyperplasia of the

thyroid gland,' but that young, growing rats do not He concluded further, that 'rats kept under unhygienic conditions developed hyperplasia of the thyroid if given a standard diet of bread and milk', and 'that unhygienic conditions *plus* a high protein diet bring about a higher degree of hyperplasia in the adult rat than either factor taken alone' (Buiget, 1917) These conclusions were based mainly on the size of the thyroid as determined by weight He used the term 'thyroid hyperplasia' to signify 'thyroid enlargement'

Lieut-Colonel C Newcomb has subjected Buiget's results to statistical examination He finds that the difference in weight of the thyroids of rats fed on Buiget's standard diet and on the high protein diets are not significant, whether the rats were adults or young and growing Even if the difference had been significant it could not with certainty have been attributed to the high protein Buiget's control animals were fed on a standard diet of bread (white bread presumably) and milk, those in which he desired to observe the effects of high protein on the thyroid gland were fed on liver or muscle mixed with a little oatmeal or bread crumbs Neither of these diets is complete, and they differ from each other not only with respect to their content of protein but with respect to their content of fats, carbohydrates, mineral salts and vitamins His observations with regard to the effects of unhygienic conditions are, on the other hand, statistically significant They indicate that whether the diet was one of bread and milk, of liver, or of lean beef and oatmeal, rats fed upon them had larger thyroids when kept under unhygienic conditions than when kept under hygienic conditions In this respect his observations are in agreement with my own (McCarrison, 1914, 1928*a* and 1930*a*) They emphasize the importance of rigidly excluding the goitrogenic influence of 'dirt' in all experiments designed to determine the effect on the thyroid gland of deficiency or excess of any food constituent

In view of the proven goitrogenic influence of 'dirt' (McCarrison, 1930*a*), of the 'incomplete' nature of the experimental diets hitherto used in studying the effect of high protein on the thyroid gland, and of the importance, as shown by recent work in this laboratory (McCarrison, 1927, 1928*b* and 1930*b*), of an insufficient supply of fat-soluble vitamins in favouring the genesis of a certain type of goitre, it seemed desirable to reconsider the question of the effect of high protein on the thyroid gland under varying conditions of vitamin and iodine-supply, the factor of unhygienic conditions of life being rigidly excluded throughout the investigation

The experimental diets

It is not possible to devise a series of experimental diets in which the protein-content is the only variable, for as the proportion of protein is increased that of some other constituent or constituents is correspondingly reduced It was decided, therefore, to arrange that certain essentials should be present in adequate quantity and constant in amount, variations being made only in the

amounts of protein, carbohydrate, iodine and fat-soluble vitamins To this end a basal diet* of the following composition was used —

White flour	80 parts
Olive oil	8 „
Salt-mixture	5 „
Cod-liver oil	2 „
Dried yeast	5 „
Distilled water	*ad libitum*

[The salt-mixture (McCollum) consisted of calcium lactate, 39 grammes, calcium phosphate, 16·2 grammes, potassium phosphate, 28·62 grammes, iron citrate, 3·54 grammes, sodium chloride, 5·19 grammes, magnesium sulphate 7·79 grammes, and sodium phosphate, 10·41 grammes.]

Variations of the protein-carbohydrate-content of this diet were made by replacing 20, 40, 60 or 80 parts of the white flour by an equivalent amount of 'meat-residue' (McCarrison, 1926) or of casein In the first experiment meat-residue was the source of the protein for the first 69 days, and casein (not vitamin-free) its source during the remaining 137 days, in the second, meat-residue was used for the first 10 days and casein for the remaining 93 days Difficulty in obtaining supplies of casein were responsible for this change in the source of the protein In the third and fourth experiments casein was used throughout

Variations in the iodine-content of the diets were made by adding potassium iodide (0·5 gramme) to, or withholding it from, the salt-mixture, as well as by giving or not giving cod-liver oil, which is a rich source of iodine Thus, in the first experiment the rats, receiving diets of varying protein-content, had *iodine added to the salt-mixture*, while *in the other three experiments* this addition was not made In the first and second experiments cod-liver oil formed part of the diets, in the third and fourth it did not The iodine-content of the diets in the four experiments thus ranged from very high to relatively low

Similarly, variations were made in the vitamin A and D-content of the diets In the first two experiments both of these vitamins were provided as cod-liver oil, in the third they were provided, in lesser amount, as 'Radiostoleum' (B D H) dissolved in hydrogenated vegetable oil (1 drop of radiostoleum to 5 c c of the oil) In the fourth experiment neither cod-liver oil nor radiostoleum was given, the animals were thus dependent, for their supply of fat-soluble vitamins, on the casein, the olive oil and the sesame oil in the diet

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*Vitamin C was not included in this diet, as rats seem to thrive quite well without it, its supply in the form of orange juice would have involved the introduction with it of a certain amount of the vitamins (A and B) contained in the juice

This supply was small, but in so far as vitamin A was concerned it was rather more, judging from the incidence of bacterial infections, than was provided by the small amount of radiostoleum given in the third experiment

The diets used in the four experiments were thus as follows —

1st experiment (16-3-29 *to* 8-10-29)

Diet	I	II	III	IV	V
Meat-residue or casein	0	20	40	60	80
White flour	80	60	40	20	0
Olive oil	8	8	8	8	8
Cod-liver oil	2	2	2	2	2
Salt-mixture (*with* K I)	5	5	5	5	5
Dried yeast	5	5	5	5	5
Distilled water	*ad libitum*				

2nd experiment (3-7-29 *to* 14-10-29)

Diet	I	II	III	IV	V
Meat-residue or casein	0	20	40	60	80
White flour	80	60	40	20	0
Olive oil	8	8	8	8	8
Cod-liver oil	2	2	2	2	2
Salt-mixture (*without* K I)	5	5	5	5	5
Dried yeast	5	5	5	5	5
Distilled water	*ad libitum*				

3rd experiment (16-12-29 *to* 21-2-30)

Diet	I	II	III	IV	V
Casein	0	20	40	60	80
White flour	80	60	40	20	0
Hydrogenated vegetable oil	8	8	8	8	8
Radiostoleum solution	2	2	2	2	2
Salt-mixture (*without* K I)	5	5	5	5	5
Dried yeast	5	5	5	5	5
Distilled water	*ad libitum*				

4th experiment (23-11-29 *to* 20-3-30)

Diet	I	II	III	IV	V
Casein	0	20	40	60	80
White flour	80	60	40	20	0
Olive oil	8	8	8	8	8
Sesame oil	2	2	2	2	2
Salt-mixture (*without* K I)	5	5	5	5	5
Dried yeast	5	5	5	5	5
Distilled water	*ad libitum*				

The experiments

The experiments were carried out in a locality (Coonoor) where goitre is not endemic Five groups of 6 young, albino rats, from non-goitrous stock, were used in each experiment, the sexes being as far as possible equally distributed in all groups and the aggregate body-weight of each group being the same Each group was made up of animals from different litters Throughout the experiments the most scrupulous attention was paid to cleanliness

The first experiment, carried out during the spring and summer of 1929, lasted 206 days when the surviving animals were killed by drowning Two animals (Nos 2374 and 2376) died during its course, these excluded The results of the experiment are set out in Table I, the weight-curves of the animals are shown in Figs 1 to 5

TABLE I

Giving details and results of the first experiment in which the diets were rich in iodine and vitamin A

1	2	3	4	5	6	7	8	9	10	11
Diet	Number of rat	Sex	Original body-weight gs	Days under experiment	Cause of death	Final body-weight gs	Weight of thyroid mg	Standard weight of normal thyroid (Coonoor rats) mg	Weight of thyroid per 100 gs body-weight mg	Average weight of thyroid per 100 gs body-weight mg
I Animal protein 0 per cent	2381	M	35	206	Killed	228	116	182	51	5·90
	2382	F	32	206	Do	169	99	112	58	
	2383	M	36	206	Do	229	113	182	49	
	2384	F	34	206	Do	132	88	116	67	
	2385	M	32	206	Do	251	140	197	56	
	2386	F	35	206	Do	141	103	123	73	
II Animal protein 20 per cent	2357	M	32	206	Do	288	142	222	42	5·93
	2358	F	34	206	Do	151	100	132	65	
	2359	M	32	206	Do	266	111	207	41	
	2360	F	35	206	Do	151	118	130	78	
	2361	M	39	206	Do	222	126	177	57	
	2362	F	32	206	Do	128	94	114	73	

III Animal protein 40 per cent	2363	M	35	206	Do	217	10·4	17·4	4·8	5·82
	2364	F	32	206	Do	153	10·4	13·1	6·8	
	2365	M	33	206	Do	193	10·9	15·8	5·6	
	2366	F	35	206	Do	131	9·2	11·5	7·0	
	2367	M	34	206	Do	263	14·0	20·5	5·3	
	2368	M	35	206	Do	195	10·6	16·0	5·4	
IV Animal protein 60 per cent	2369	F	31	206	Do	163	10·6	13·8	6·5	6·22
	2370	F	36	206	Do	160	11·0	13·6	6·9	
	2371	M	37	206	Do	220	11·8	17·6	5·4	
	2372	F	35	206	Do	145	9·0	12·5	6·2	
	2373	M	34	206	Do	218	13·4	17·5	6·1	
	2374	M	31	35	Enteritis	85	excluded			
V Animal protein 80 per cent	2375	M	37	206	Killed	194	9·9	15·9	5·1	5·94
	2376	F	32	5	Injury	excluded				
	2377	M	33	206	Killed	202	9·4	16·4	4·6	
	2378	F	31	206	Do	207	12·0	16·8	5·8	
	2379	F	32	206	Do	132	11·2	11·6	8·5	
	2380	F	39	206	Do	164	9·4	13·9	5·7	

It will be noted that the basal diet without added protein admitted of good growth especially in males (M), that the best growth was exhibited by males receiving 20 per cent of animal protein, and the next best by males

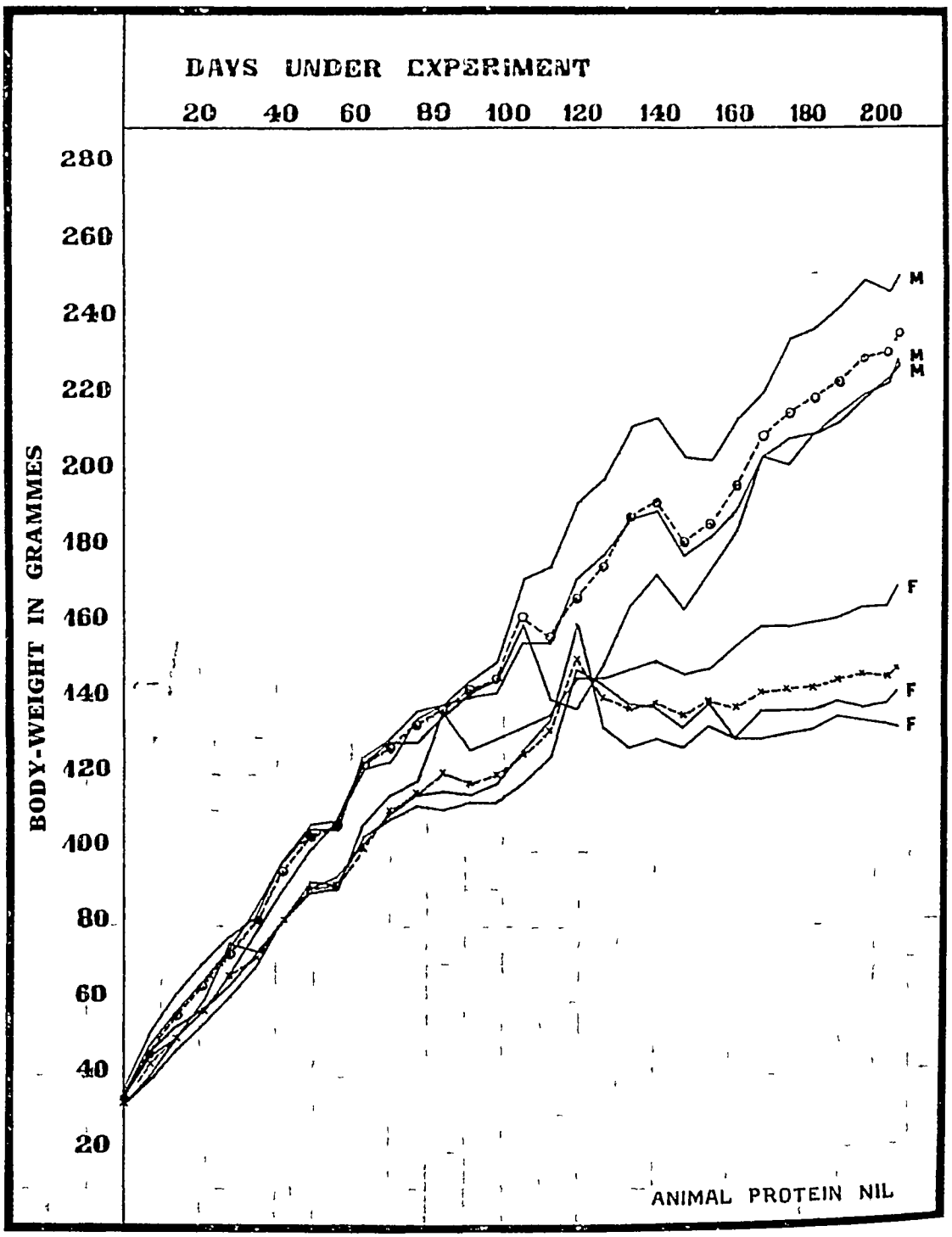

Fig 1—Showing the weight-curves of 3 male (M) and 3 female (F) rats fed on the diet containing no animal protein Average weight-curves shown as interrupted lines

receiving none, the growth of males receiving 40, 60 and 80 per cent being on a somewhat lower level In all groups the rate of growth of females (F) was well below that of males and varied very little in the different groups

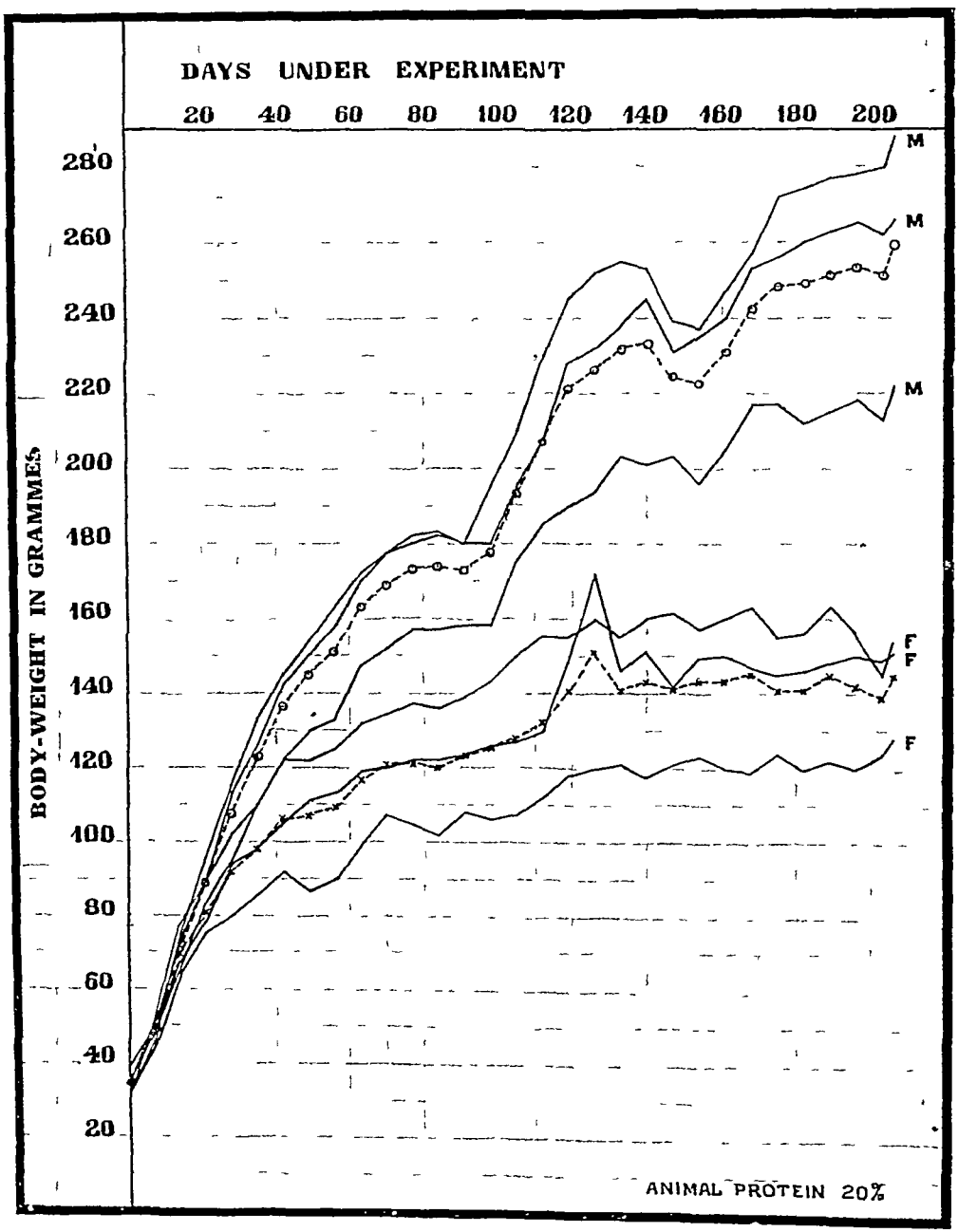

Fig 2—Showing the weight-curves of 3 male (M) and 3 female (F) rats fed on the diet containing 20 per cent of animal protein Average weight-curves shown as interrupted lines

So far then as the growth of the rats in the several groups was concerned, it did not vary so greatly as to be reflected in the weights of the thyroid glands An examination of Table I shows that in all 5 groups the weights of the thyroid

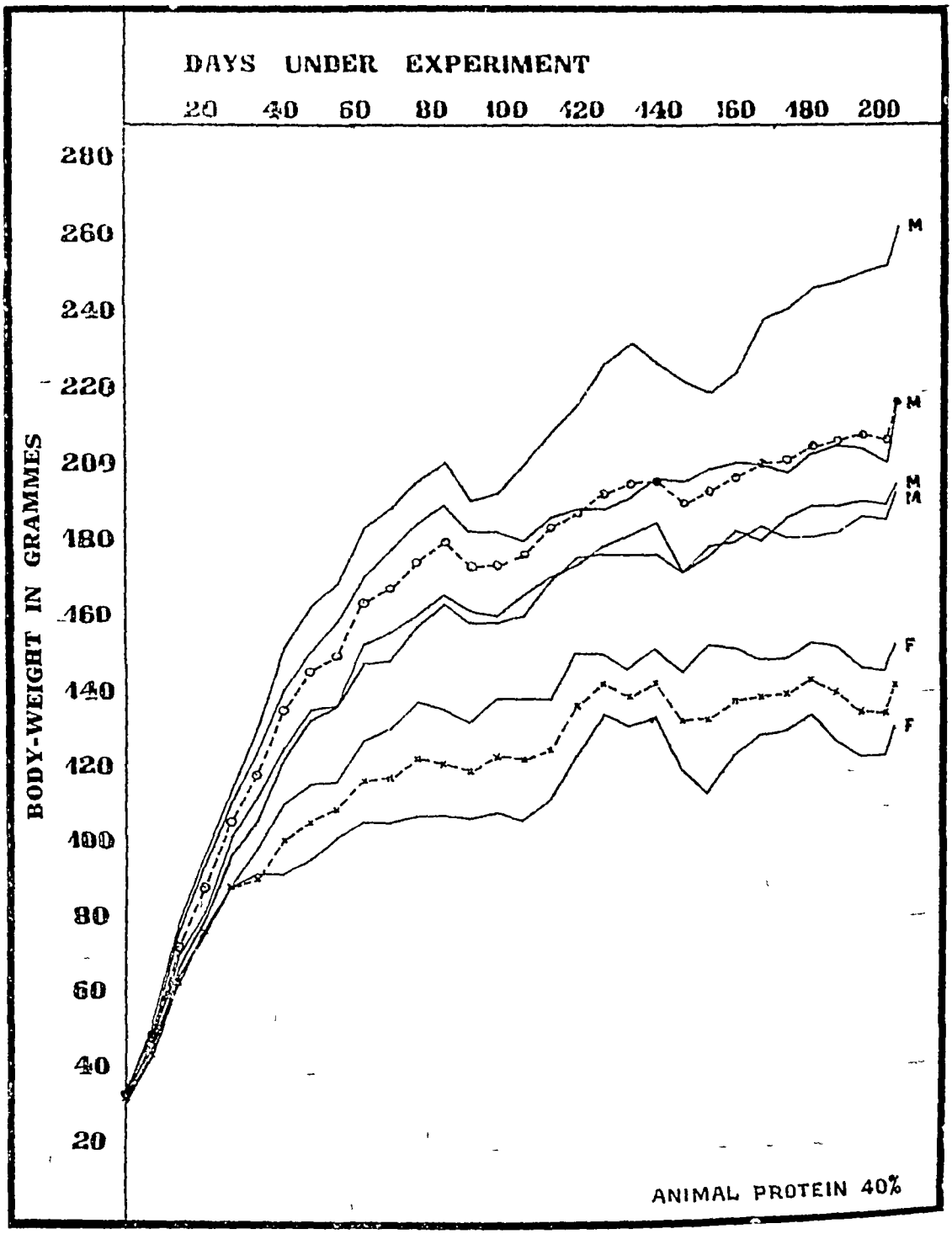

Fig 3—Showing the weight-curves of 4 male (M) and 2 female (F) rats fed on the diet containing 40 per cent of animal protein Average weight-curves shown as interrupted lines

were invariably less than the normal standards, there was, however, no significant difference in the average size of the gland in the five groups Growing rats kept under hygienic conditions did not, therefore, develop thyroid enlarge-

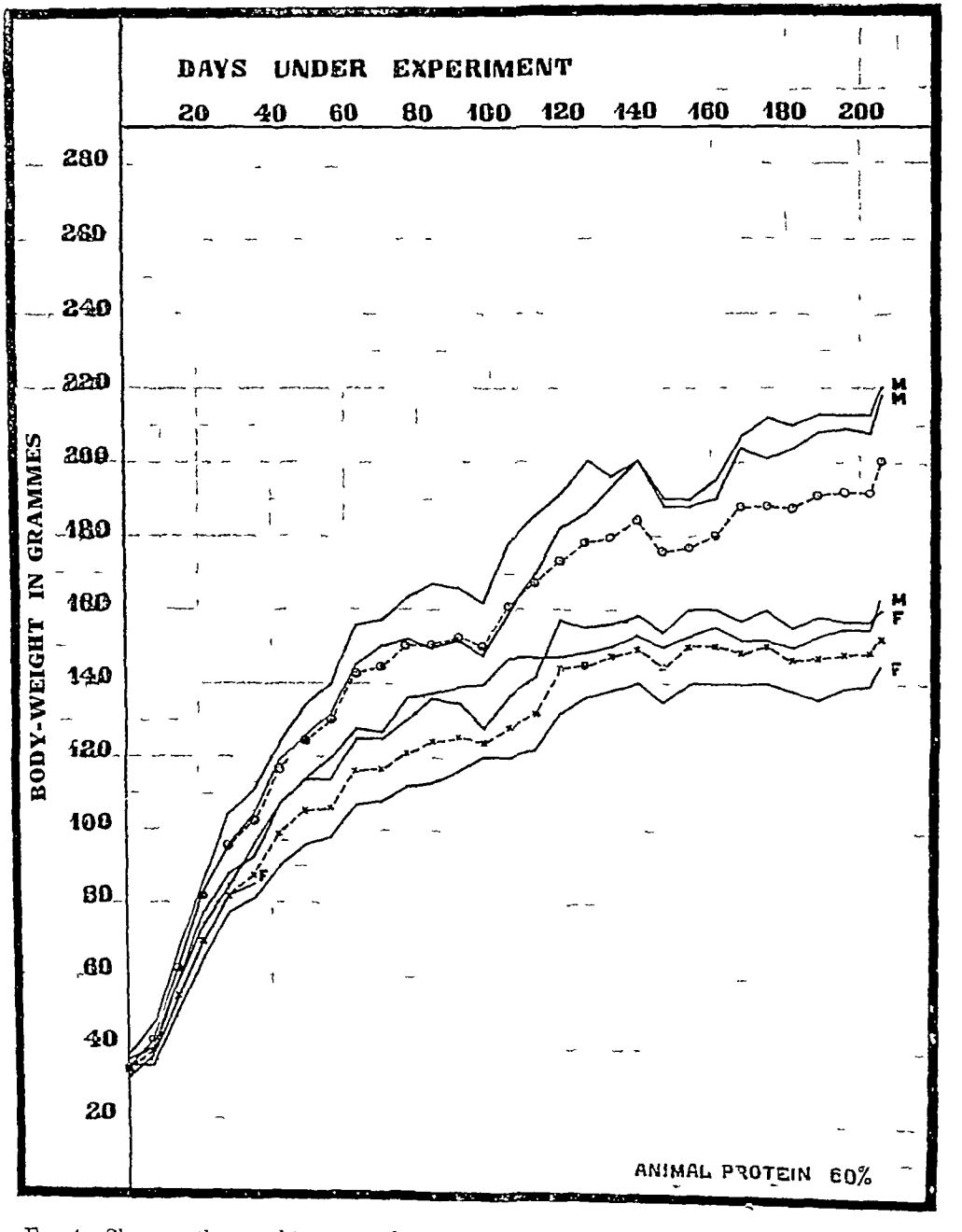

Fig 4—Showing the weight-curves of 3 male (M) and 2 female (F) rats fed on the diet containing 60 per cent of animal protein Average weight-curves shown as interrupted lines

ment, within a period of 206 days, when fed on high protein diets containing relatively large amounts of iodine and a sufficiency of fat-soluble vitamins

The urinary excretion of iodine by the rats in this experiment ranged from 125,000 to 291,700 γ per litre

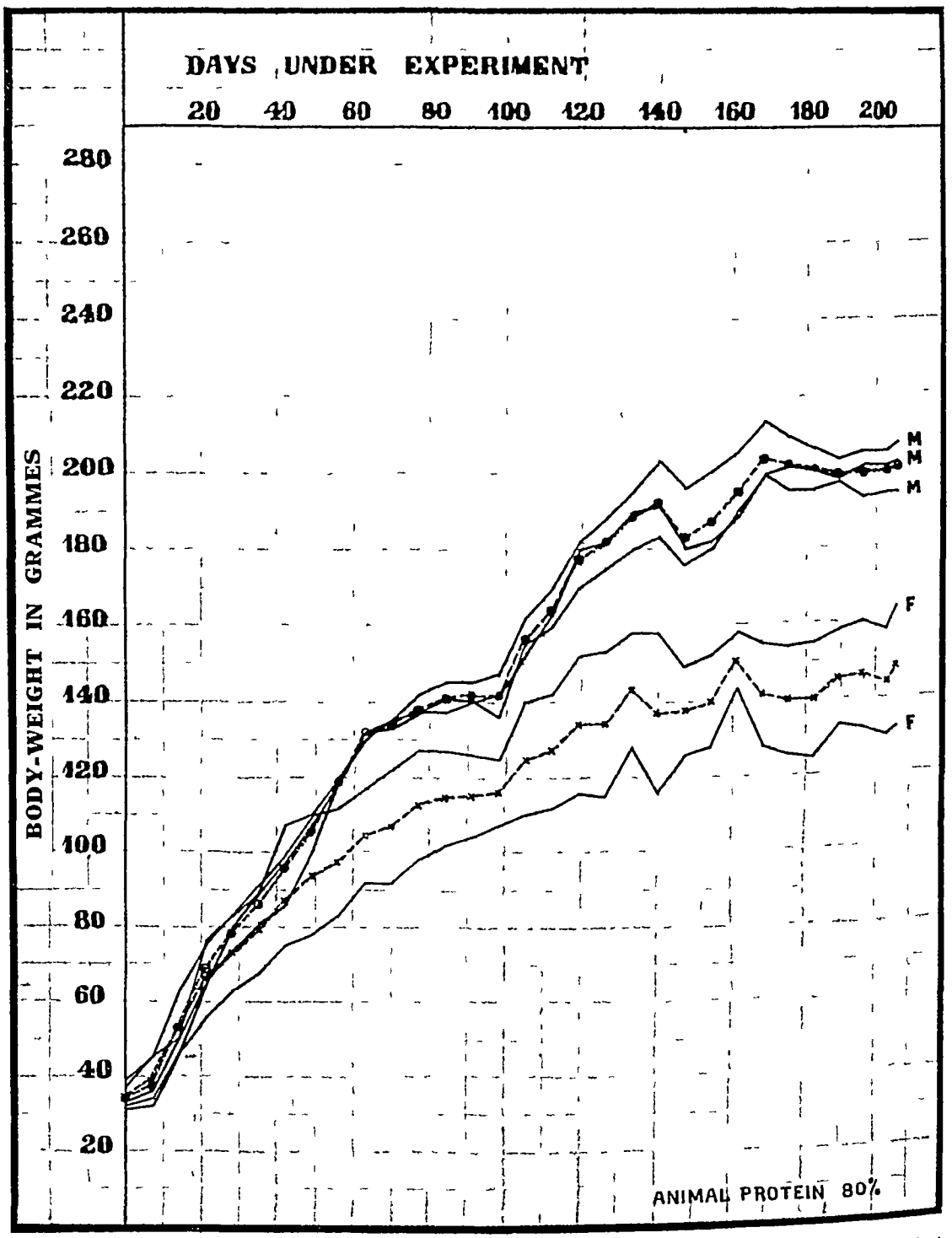

Fig 5.—Showing the weight-curves of 3 male (M) and 2 female (F) rats fed on the diet containing 80 per cent of animal protein. Average weight-curves shown as interrupted lines

The second experiment was carried out during the late summer and early autumn of 1929 Its results are set out in Table II One animal (No 2590) died during its course, the rest were killed by drowning This experiment differed little from the first except that it was of a shorter duration (103 days), the animals used were a little older, and no potassium iodide was added to the salt-mixture contained in their diets

Ten of the rats—(two in each group shown in **bold type** in Table II) were kept in metabolism cages during the last 68 days of the experiment when certain aspects of their metabolism were studied For the first 43 days of their stay in these cages they received the same diets as the other animals of their groups, but during the last 25 days their diets, instead of containing 2 parts of cod-liver oil, contained 2 parts of sesame oil in which 2/5th of a drop of radiostoleum was dissolved The object of this change was to observe its effects on the urinary excretion of iodine It is remarkable that 4 of these 10 rats had thyroids considerably larger than those of the remaining 19 The mean thyroid-weight of the 10 rats receiving radiostoleum was 15 0 mg for a mean body-weight of 158 5 gs , while the mean thyroid-weight of the other 19 rats was 10·2 mg for a mean body-weight of 147 7 gs Disregarding the differences in body-weights the difference in thyroid-weights (amounting to 4 8 mg) is large If, however, the actual thyroid-weights are compared with the weights appropriate to each given body-weight on the basis of the normal thyroid curve for Coonoor stock rats (*see* Diagram, page 656), the ten rats receiving radiostoleum show a mean excess of 1 59 mg , whereas the other 19 rats show a mean deficiency of 2·48 mg The commuted difference is thus 4 07 mg which is, statistically speaking, significant Yet I hesitate to conclude that the change from cod-liver oil to radiostoleum during the last 25 days of the experiment was responsible for the larger size of the thyroid gland in the animals receiving radiostoleum This point is more fully dealt with by Professor Madhava in his statistical note

Four of these rats (Nos 2591, 2592, 2570 and 2576) had thyroids which were significantly larger than the normal standard, all 4 were fed on diets containing 40 or less per cent of animal protein These goitres were not, therefore, related to a high content of animal protein in the diet

Excepting the above-mentioned 10 rats, whose treatment was different to that of the others, the remaining 19 had—as in the first experiment—thyroids which were smaller than the normal standard The different treatment which these 10 rats received during the last 25 days of the experiment does not affect the general result since equal numbers in each group were treated in a precisely similar way This result may be stated as follows Growing rats kept under hygienic conditions of life and fed during a period of 103 days on diets containing a high proportion of animal protein, a moderate amount of iodine and a sufficiency of fat-soluble vitamins, had thyroids which were no larger than those of rats whose diets contained little or no animal protein

The weight-curves of the animals are shown in Figs 6 to 10 It will be noted from these figures that the basal diet without added protein admitted of good growth, that the average rate of growth of males was approximately the same in those receiving no animal protein, those receiving 20 and those receiving 40 per cent The rate of growth of males receiving 60 per cent was on a somewhat lower level, while that of males receiving 80 per cent was considerably lower Females grow best when the protein-content of the diet was low

TABLE II

Giving details and results of the second experiment in which the diets were rich in vitamin A and moderately rich in iodine

1	2	3	4	5	6	7	8	9	10	11
Diet	Number of rat	Sex	Original body-weight gs	Days under experiment	Cause of death	Final body-weight gs	Weight of thyroid mg	Standard weight of normal thyroid (Coonoor rats) mg	Weight of thyroid per 100 gs body-weight mg	Average weight of thyroid per 100 gs body-weight mg
I Animal protein 0 per cent	2591	F	60	103	Killed	172	19·3	11·4	11·2	
	2592	F	53	103	Do	144	17·2	12·1	11·9	
	2593	F	57	103	Do	127	10·9	11·3	8·6	8·13
	2594	M	54	103	Do	208	12·4	16·9	5·9	
	2595	M	52	103	Do	168	11·7	14·1	6·9	
	2596	M	53	103	Do	193	11·7	15·8	6·1	
II Animal protein 20 per cent	2567	F	50	103	Do	123	7·7	11·0	6·2	
	2568	F	53	103	Do	144	9·3	12·4	6·4	
	2569	F	60	103	Do	132	9·3	11·6	7·0	6·97
	2570	F	8	103	Do	224	21·8	17·9	9·7	
	2571	M	53	103	Do	238	13·0	18·8	5·5	
	2572	M	55	103	Do	145	10·2	12·5	7·0	

III Animal protein 40 per cent	2573	F	53	103	Do	115	7·5	10·4	6·5	7·52
	2574	F	50	103	Do	146	8·5	12·5	5·8	
	2575	F	60	103	Do	140	10·0	12·2	7·1	
	2576	**F**	**60**	**103**	**Do**	**137**	**15·8**	12·0	**11·5**	
	2577	**M**	**51**	**103**	**Do**	**194**	**16·8**	15·9	**8·6**	
	2578	M	55	103	Do	190	10·6	15·6	5·6	
IV Animal protein 60 per cent	**2579**	**F**	**60**	**103**	**Do**	**142**	**12·0**	12·3	**8·4**	8·23
	2580	F	51	103	Do	118	10·0	10·7	8·5	
	2581	F	54	103	Do	110	9·9	10·1	9·0	
	2582	**M**	**59**	**103**	**Do**	**172**	***15·6***	14·4	**9·0**	
	2583	M	53	103	Do	156	10·6	13·3	6·8	
	2584	M	52	103	Do	158	12·2	13·5	7·7	
V Animal protein 80 per cent	2585	F	54	103	Do	120	11·0	10·8	9·1	8·28
	2586	F	53	103	Do	83	6·2	8·0	7·4	
	2587	F	60	103	Do	124	10·4	11·1	8·4	
	2588	**M**	**54**	**103**	**Do**	**126**	**10·0**	11·2	**7·9**	
	2589	**M**	**52**	**103**	**Do**	**142**	**12·2**	12·3	**8·6**	
	2590	M	56	26	Pneumonia	60	excluded			

The urinary excretion of iodine by the rats in this experiment ranged between 150 and 110 γ per litre when they were receiving cod-liver oil, and

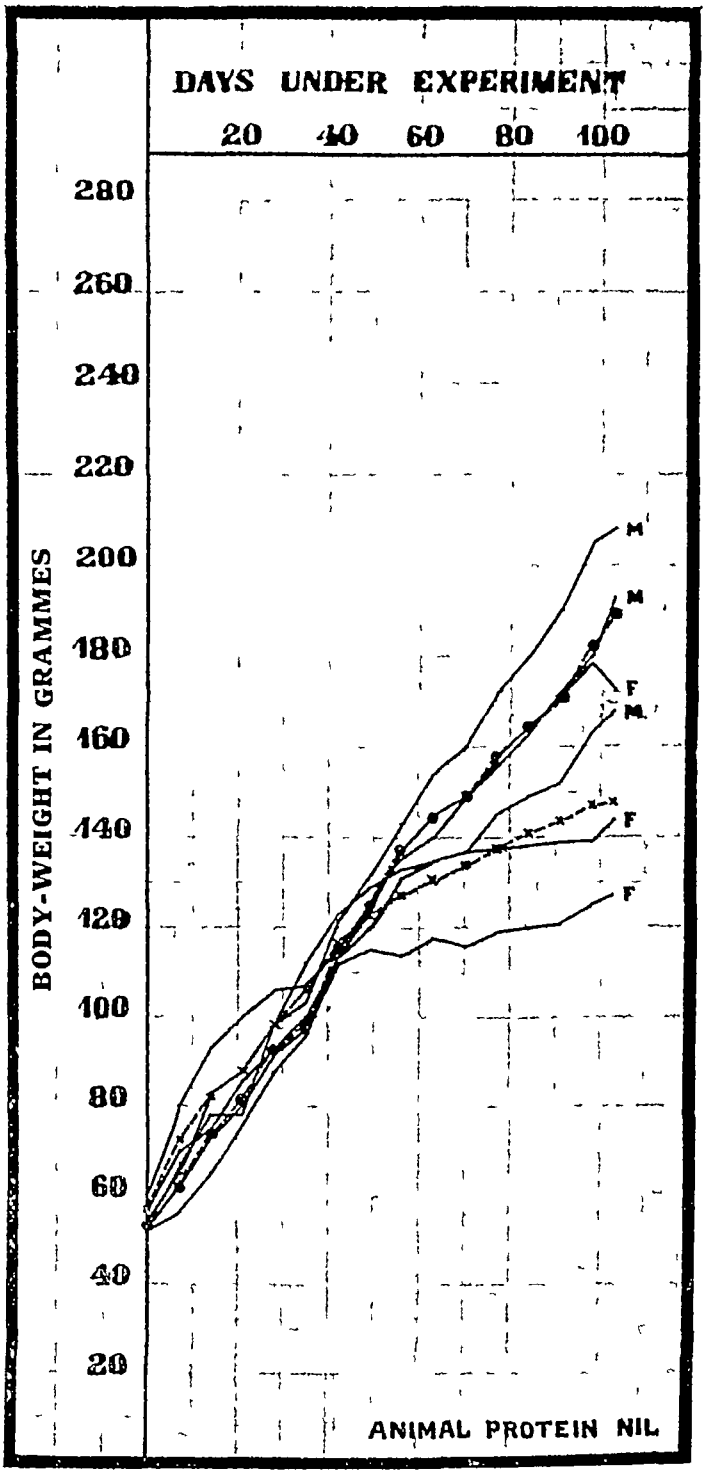

Fig 6—Showing the weight-curves of 3 male (M) and 3 female (F) rats fed on the diet containing no animal protein Average weight shown by interrupted lines

between 40 and 120 γ per litre when they were receiving radiostoleum (Table VI)

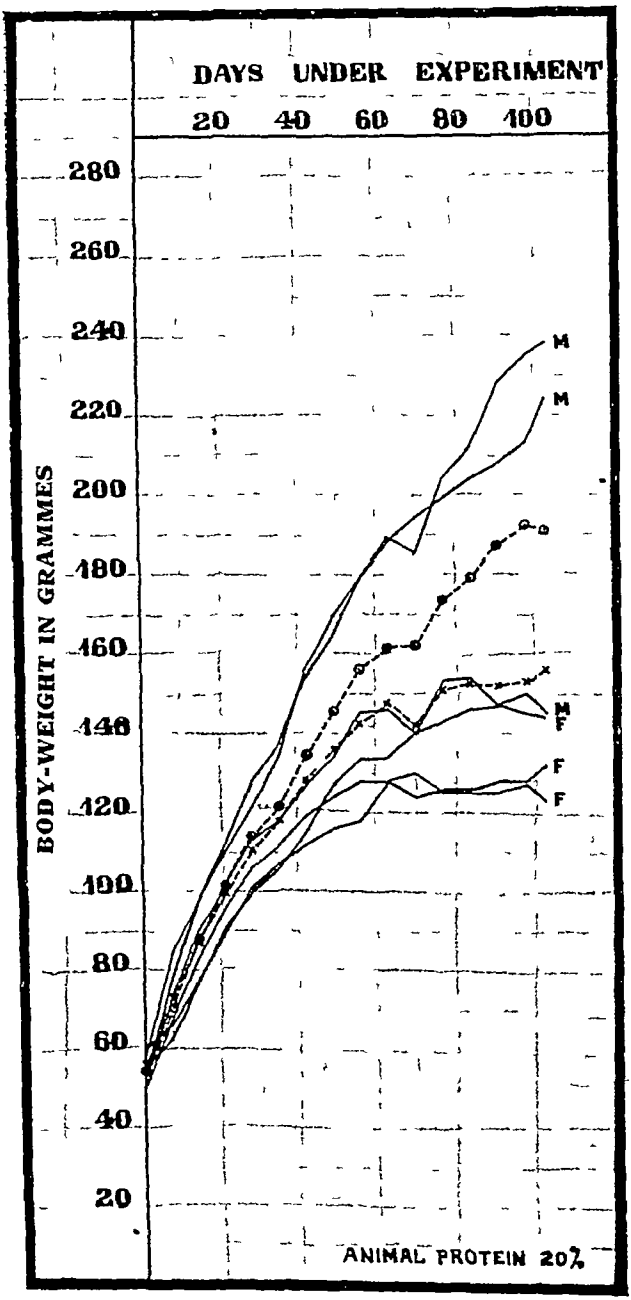

Fig 7—Showing the weight-curves of 3 male (M) and 3 female (F) rats fed on the diet containing 20 per cent animal protein Average weight shown by interrupted lines

The third experiment was carried out during the winter of 1929-30 It differed from the first two in that vitamins A and D were provided as radiostoleum, and fats as hydrogenated vegetable oil The amount of radiostoleum consumed by each animal daily was approximately 0 01 of a drop an amount

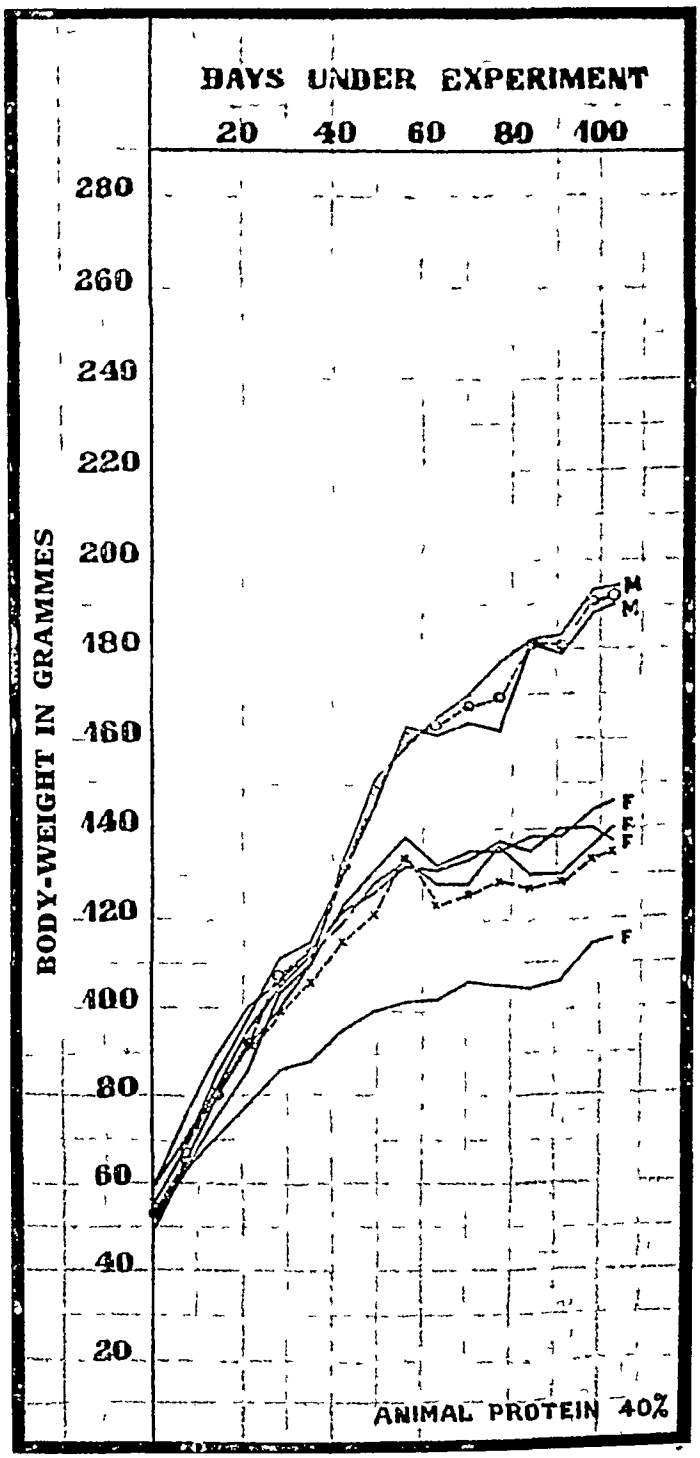

Fig 8—Showing the weight-curves of 2 male (M) and 4 female (F) rats fed on the diet containing 40 per cent animal protein Average weight shown by interrupted lines

which, judging by the diseases from which the animals suffered, was altogether insufficient for the needs of growing animals or to protect them against infection The diets in this experiment were low in iodine relative to those used in

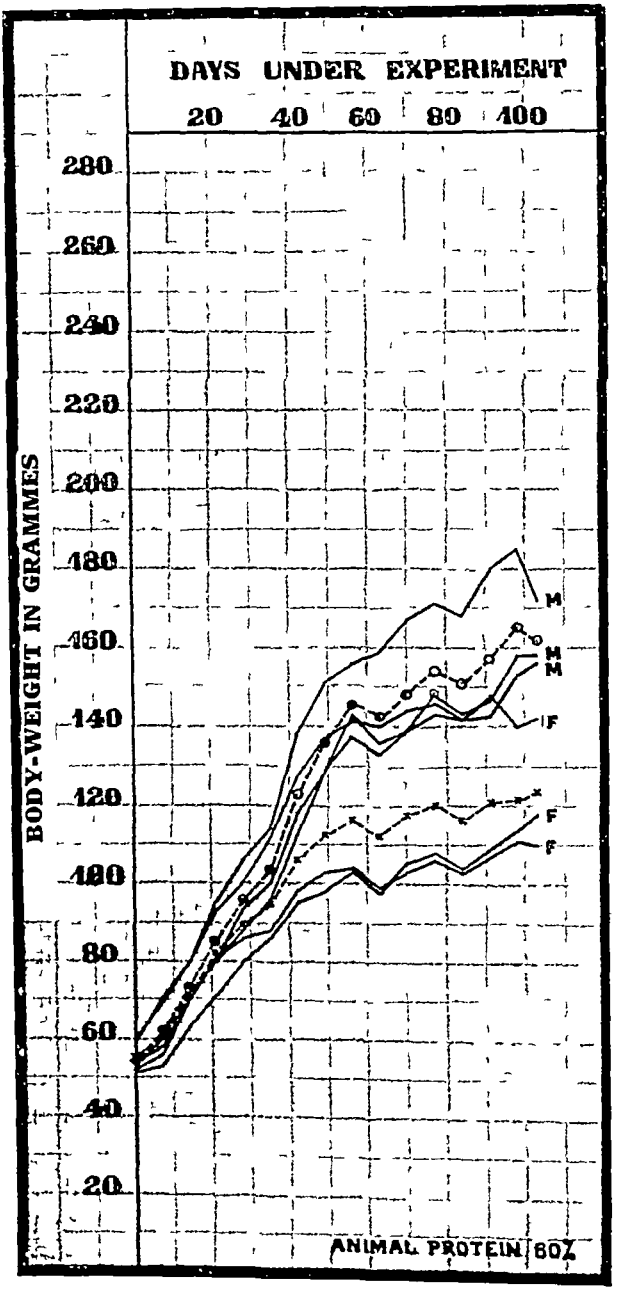

Fig 9—Showing the weight-curves of 3 male (M) and 4 female (F) rats fed on the diet containing 60 per cent animal protein Average weight-curves shown by interrupted lines

the first two experiments The urinary excretion of iodine ranged between 49 and 62 γ per litre, the average being 58 γ The experiment lasted 94 days, only three of the 30 animals surviving for that length of time The results are

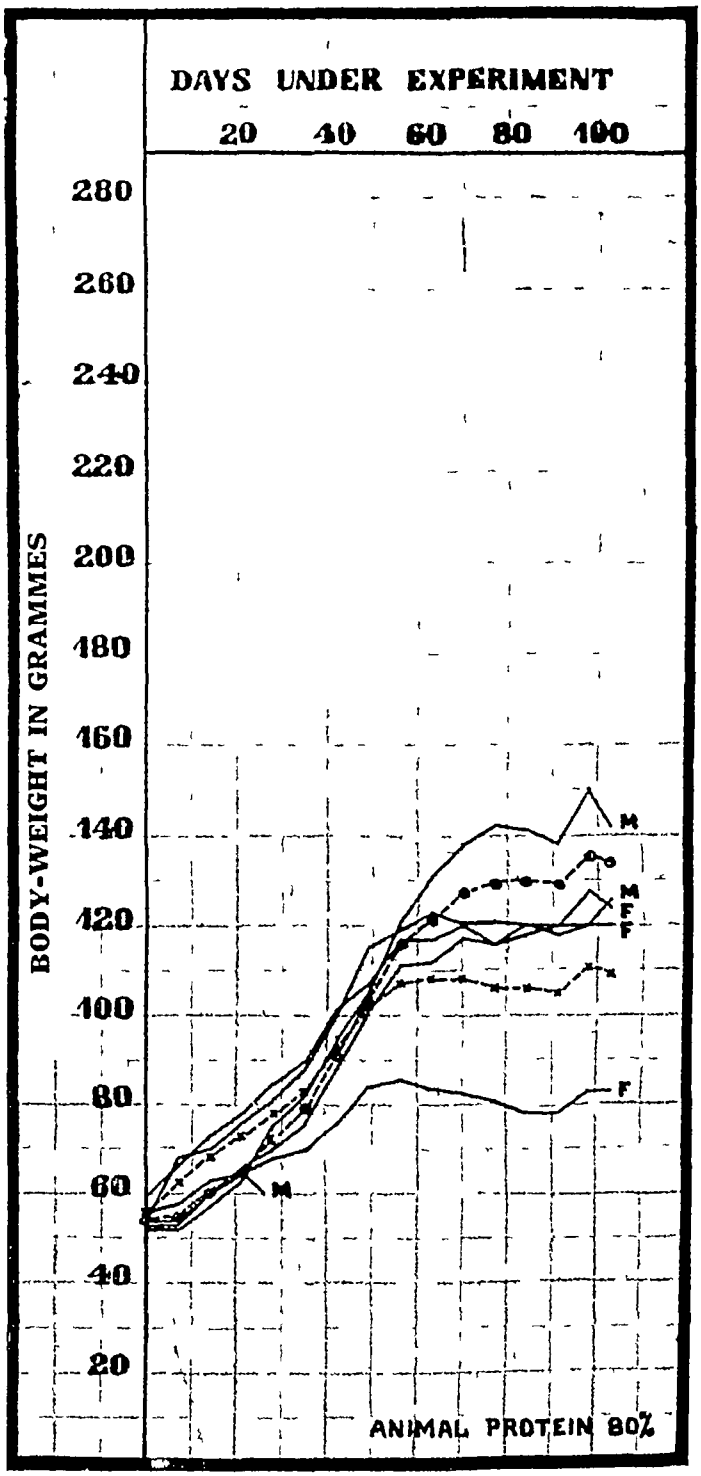

Fig 10—Showing the weight-curves of 3 male (M) and 3 female (F) rats fed on the diet containing 80 per cent animal protein Average weight-curves shown by interrupted lines

set out in Table III It will be noted from this table that the growth of the rats in all five groups was very poor and that diseases of the gastro-intestinal, pulmonary and urinary tracts were rife amongst them Table III provides a striking demonstration, when compared with Tables I and II, of the effect of insufficiency of vitamin A in causing disease of the pulmonary, the urinary and the gastro-intestinal tracts

In so far as the effects of high-protein diets on the thyroid gland were concerned, the result of this experiment did not differ from those of the previous two It may be expressed as follows —In the presence of an insufficiency of fat-soluble vitamins in the food and of a relatively low intake of iodine, a high proportion of animal protein in the diet does not cause thyroid enlargement within a period of 94 days in young rats living under hygienic conditions The higher concentration of protein had, however, the effects of causing the animals to die earlier from diseases of bacterial origin

Emphasis is here laid on the length of time the experiment lasted, for, as shown in another place (McCarrison, 1930*c*), if the animals had survived longer some of them might have developed goitre not because of the high protein but because of the deficiency in fat-soluble vitamins in their diets As it was there were two animals (Nos 2831 and 2837) whose thyroids weighed significantly more than the normal standard But neither of these were fed on diets containing excessive amounts of protein, one having received '0 per cent' and the other '20 per cent' protein Such enlargements of the thyroid gland as did occur were not, therefore, associated with a high content of protein in the vitamin-deficient diets

The fourth experiment was carried out during the winter of 1929-30 It differed from the others in that no fat-soluble vitamin other than that contained in the casein, the olive oil and the sesame oil, was given to the animals The results are set out in Table IV

It will be noted that the average period of survival of the rats in this experiment was considerably longer than that of the animals in the third experiment in which a small amount of vitamin A was provided as radiostoleum Eleven animals survived for the full period of the experiment—117 days So far as growth, period of survival and incidence of disease were concerned, the diets containing 0 per cent and 20 per cent casein was the best, while those containing 60 and 80 per cent were the worst It will be noted that the period of survival of animals ingesting much protein was considerably shorter than that of rats ingesting less This experiment, like the third, is a striking illustration of the effect of vitamin A-deficiency in rendering rats susceptible to bacterial infection of mucous surfaces The high incidence of cystitis, without accompanying stone-formation, is interesting, it would seem to indicate that deficiency of vitamin A in association with a high content of animal protein in the diet is favourable to the production of this condition

In this experiment, also, high-protein *per se* did not cause thyroid enlargement

TABLE III

Giving details and results of the third experiment in which the diets were poor in vitamin A and relatively low in iodine

1	2	3	4	5	6	7	8	9	10	11	12
Diet	Number of rat	Sex	Original body-weight gs	Days under experiment	Average period of survival days	Cause of death	Final body-weight gs	Weight of thyroid mg	Standard weight of normal thyroid (Coonoor rats) mg	Weight of thyroid per 100 gs body-weight mg	Average weight of thyroid per 100 gs body-weight mg
I Animal protein 0 per cent	2831	M	53	87	66	?	75	10·8	7·4	14·4	9·8
	2832	M	48	90		Stone	81	5·8	7·8	7·2	
	2833	M	57	48		?	75	5·8	7·4	7·7	
	2834	F	47	94		Killed	79	7·2	7·7	9·1	
	2835	F	52	41		Bi-pneumonia	58	5·0	6·1	8·6	
	2836	F	48	34		Pneumonia	53	6·2	5·5	11·7	
II Animal protein 20 per cent	2837	M	57	25	61	?	76	12·2	7·5	16·0	10·2
	2838	M	57	43		Inanition	58	6·8	6·1	11·7	
	2839	M	45	75		Pneumonia Cystitis	77	5·6	7·6	7·3	
	2840	F	52	75		Stone	85	8·8	8·2	10·3	
	2841	F	49	93		Do	59	5·0	6·1	8·5	
	2842	F	45	56		Cystitis pyonephrosis	65	4·8	6·6	7·4	

III Animal protein 40 per cent	2843	M	53	6	71	Inanition	42	excluded			8·5
	2844	M	46	49		Gastro-intestinal disease	68	5·4	6·9	7·9	
	2845	M	57	94		Killed	123	10·4	11·0	8·5	
	2846	F	47	60		Stone	60	4·8	6·2	8·0	
	2847	F	50	61		Cystitis	89	8·6	8·6	9·7	
	2848	F	52	94		Killed	96	8·2	9·0	8·5	
IV Animal protein 60 per cent	2849	M	50	72	42	Inanition	55	3·8	5·7	6·9	9·2
	2850	M	53	38		Do	48	4·8	5·1	10·0	
	2851	M	48	34		Stone	55	5·4	5·7	9·8	
	2852	F	49	36		Kidney disease	48	4·6	5·1	9·6	
	2853	F	48	46		Cystitis	62	5·2	6·3	8·4	
	2854	F	57	27		Pneumonia	48	5·2	5·1	10·8	
V Animal protein 80 per cent	2855	M	54	67	49	Cystitis pneumonia	37	3·0	4·1	8·1	10·2
	2856	M	54	44		Inanition	49	5·0	5·2	10·2	
	2857	M	46	51		Cystitis	37	3·8	4·1	10·3	
	2858	F	48	45		Pneumonia cystitis	47	5·2	5·1	11·0	
	2859	F	56	16		Pneumonia	44	excluded			
	2860	F	47	40		Gastro-intestinal disease	42	4·4	4·5	10·5	

TABLE IV

Giving details and results of the fourth experiment in which the diets were relatively low in vitamin A and in iodine

1	2	3	4	5	6	7	8	9	10	11	12
Diet	Number of rat	Sex	Original body-weight gs	Days under experiment	Average period of survival days	Cause of death	Final body-weight gs	Weight of thyroid mg	Standard weight of normal thyroid (Coonoor rats) mg	Weight of thyroid per 100 gs body-weight mg	Average weight of thyroid per 100 gs body-weight mg
I Animal protein 0 per cent	2801	F	43	117	106	Killed	105	7·2	9·7	6·9	8·1
	2802	F	46	64		Hydro-thorax	114	8·2	10·5	7·2	
	2803	F	42	117		Killed	105	11·8	9·7	11·2	
	2804	M	40	117		Do	117	10·0	12·7	6·8	
	2805	M	40	14		Do	54	excluded			
	2806	M	51	117			130	10·6	11·5	8·2	
II Animal protein 20 per cent	2807	F	41	45	99	Stone	68	8·0	6·9	11·8	9·0
	2808	F	43	117		Killed	110	7·2	10·1	6·5	
	2809	F	49	117		Do	102	8·2	9·5	8·0	
	2810	M	48	117		Do	127	9·0	11·1	7·1	
	2811	M	41	117		Do	131	16·2	11·8	12·1	
	2812	M	40	79		Pneumonia	85	7·1	8·2	8·7	

III Animal protein 10 per cent	2813	F	16	109	96	Cystitis	74	8·0	7·3	10·8	7·9
	2814	F	43	72		Do	65	4·6	6·6	7·1	
	2815	F	47	71		Do	70	4·4	7·0	6·3	
	2816	M	46	117		Killed	86	7·8	8·3	9·0	
	2817	M	40	117		Do	84	5·4	8·1	6·4	
	2818	M	40	93		Enteritis	72	5·6	7·1	7·8	
IV Animal protein 60 per cent	2819	F	47	61	72	Inanition	42	4·0	4·5	9·5	8·5
	2820	F	45	117		Killed	66	6·4	6·7	9·7	
	2821	F	40	38		Pneumonia and stone	41	3·2	4·4	7·8	
	2822	M	50	96		Pneumonia	79	6·4	7·7	8·1	
	2823	M	40	63		*Inanition*	41	3·2	4·4	7·8	
	2824	M	40	60		Cystitis	55	4·6	5·7	8·4	
V Animal protein 80 per cent	2825	F	50	82	72	Abscess	52	4·8	5·4	9·2	10·3
	2826	F	46	91		Pneumonia	54	6·4	5·7	11·9	
	2827	F	41	69		Cystitis	37	3·6	4·1	9·7	
	2828	M	43	14		Inanition	32		excluded		
	2829	M	41	71		Do	39	4·6	4·2	11·8	
	2830	M	41	49		Gastro-intestinal disease	39	3·4	4·2	8·7	

The urinary excretion of iodine by the rats in this experiment ranged between 70 and 90 γ per litre, the average being 77·6 γ.

Discussion of results.

For purposes of interpreting the results of these experiments, the five diets are divided into two categories 'high protein diets,' containing 40 or more per cent of animal protein, and 'low protein diets,' containing 20 or less per cent of protein. These are further sub-divided into 'complete' and 'incomplete' diets: 'complete,' when they contained a sufficiency of fat-soluble vitamins and iodine, 'incomplete,' when they contained an insufficiency of fat-soluble vitamins and relatively little iodine. Arranged according to these categories the weights of the thyroids have been plotted out (Figs. 11, 12 and 13) against the

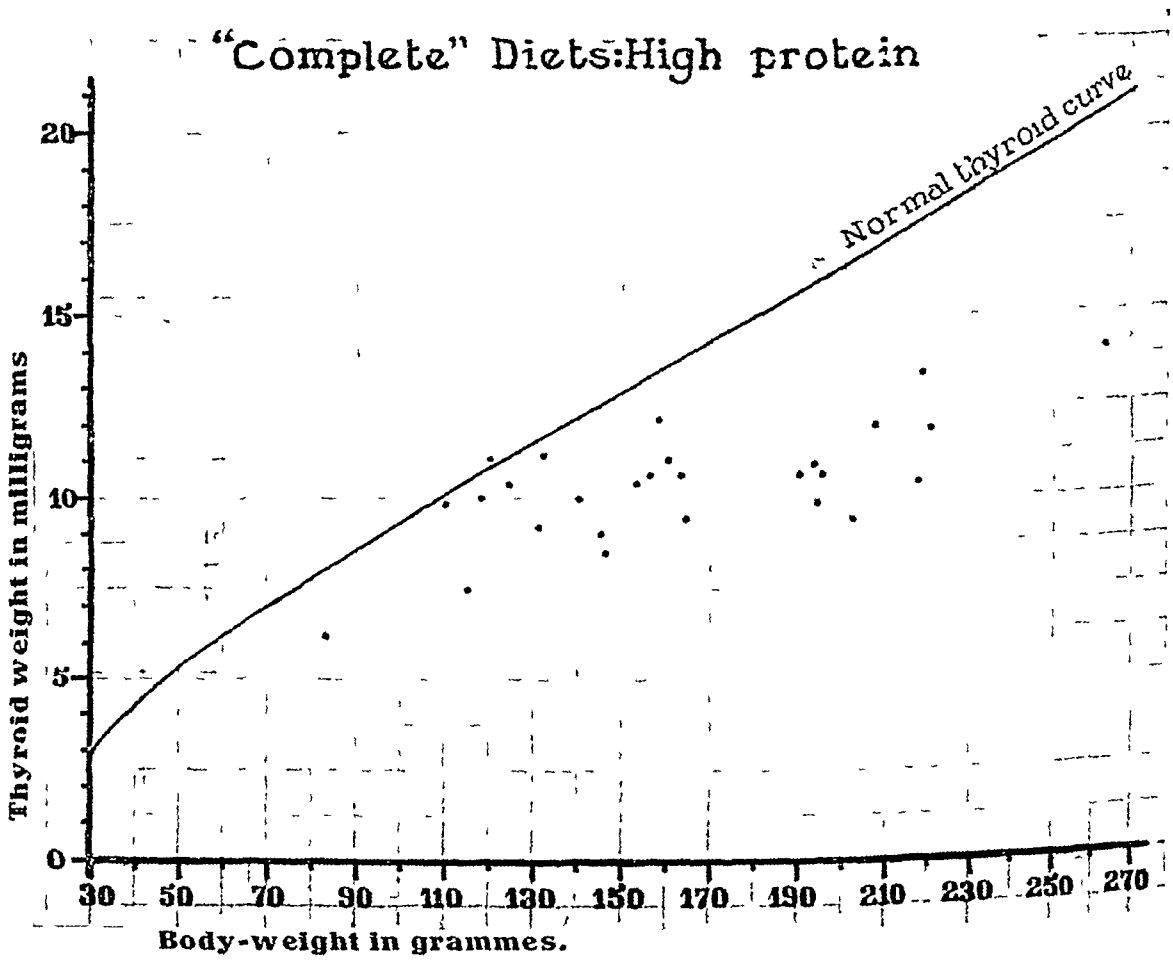

Fig. 11.—Showing that the weights of thyroids of rats fed on complete, high-protein diets fell, with few exceptions, below the normal standard.

curve, prepared by Professor Madhava, for the normal thyroids of well-fed, Coonoor stock rats. In preparing Figs. 11 and 12, the ten rats have been excluded in whose diet cod-liver oil was replaced, during the last 25 days of the second experiment, by a small and insufficient amount of radiostoleum.

An examination of Figs 11 and 12 shows that the thyroid glands of rats fed on the so-called 'complete' diets were smaller than normal, with few exceptions the glands in both the high and low protein categories fell below the normal standard of weight It is notable also that the weightier the animals (i e, the longer they subsisted on the experimental diets) the further did the weights of their thyroids fall below the normal standard There was, however, no appreciable difference in the weights of the thyroids in the two categories High protein *per se* did not cause enlargement of the thyroid gland, nor was the diminution in size of the thyroid the result of high protein ingestion, since it occurred equally in rats fed on high and on low protein diets The composition of the 'complete' diets was such as to cause the thyroid gland to be

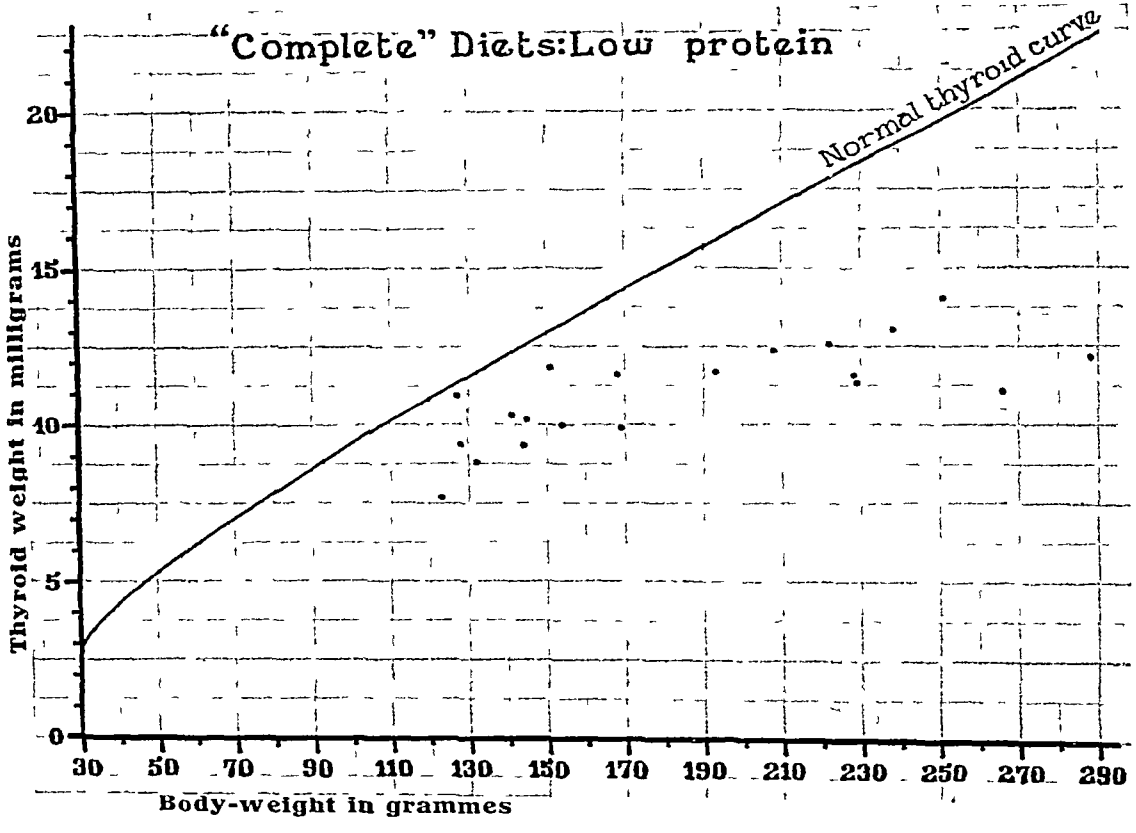

Fig 12—Showing that the weights of the thyroids of rats fed on complete, low-protein diets fell below the normal standard

smaller than normal, and to this effect the protein-content of the diets did not appreciably contribute

An examination of Fig 13, which relates to the thyroids of rats fed on 'incomplete' high and low protein diets, also reveals that high protein *per se*

did not cause thyroid enlargement, on the contrary, the thyroids of rats fed on the 'incomplete' low protein diets were larger, on the average, than those of rats fed on the 'incomplete' high-protein diets. Amongst the former there were four that were definitely goitrous, while 8 out of a total of 23 were above the normal standard of weight.

As between complete' (Figs. 11 and 12) and 'incomplete' (Fig. 13) diets whether their content of animal protein was high or low, there is a distinct difference: in general the thyroids of rats fed on incomplete diets tended to be larger than those of rats fed on complete diets, while there was a definite tendency to goitre-formation in rats fed on incomplete diets containing relatively little animal protein. It would appear, therefore, that insufficiency of fat-soluble vitamins in association with a low content of animal protein (or a high content of carbohydrate) in the diet is definitely favourable to goitre-formation.

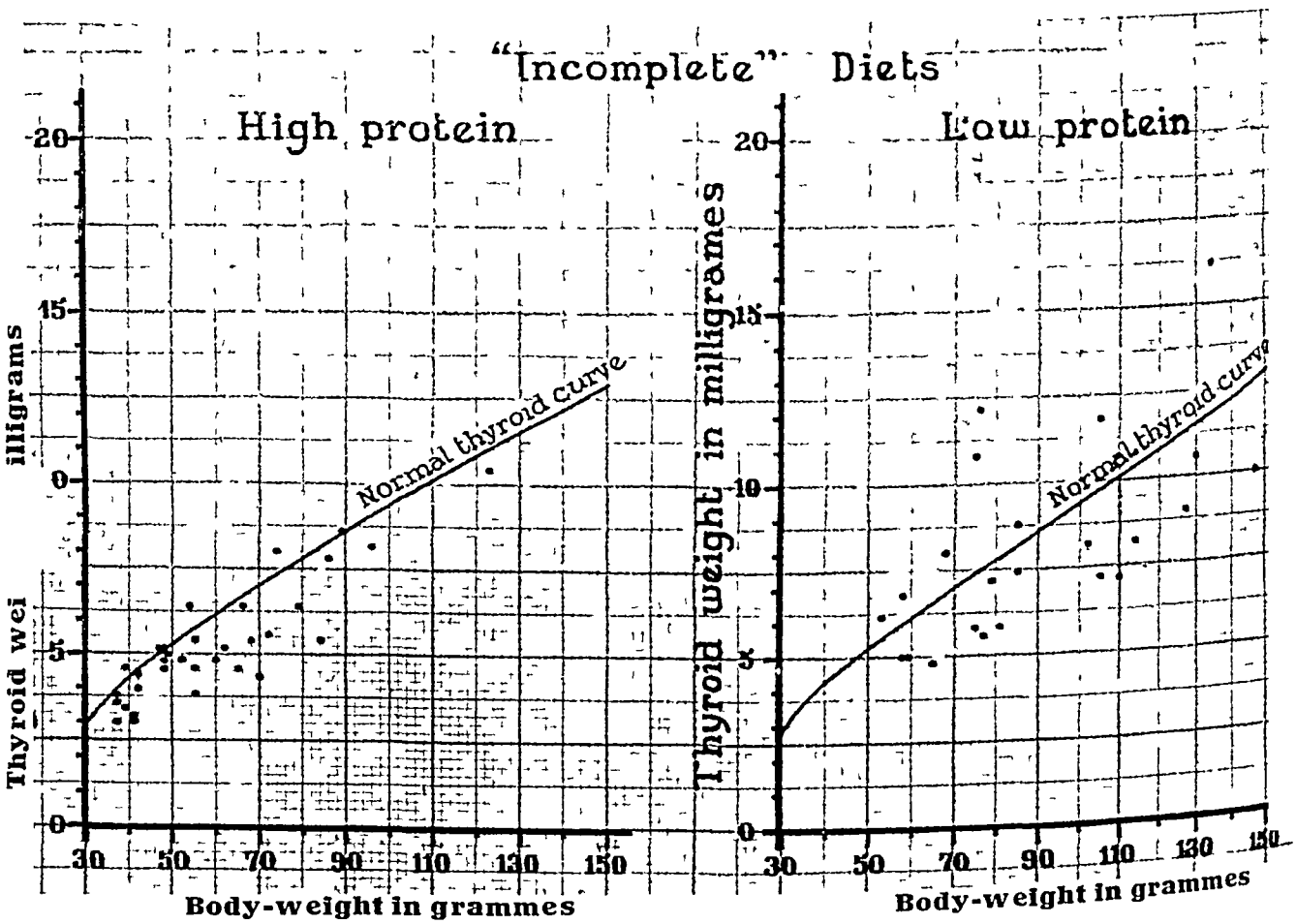

Fig. 13.—Showing that the weights of the thyroids of rats fed on incomplete high and low protein diets fell, for the most part below the normal standard. Note the tendency to goitre-formation in rats fed on incomplete low-protein diets.

Figs 11, 12 and 13 would equally well apply to the thyroids of rats whose diets were rich (Figs 11 and 12) and relatively poor (Fig 13) in iodine, since the constitution of the experimental diets was such that those rich in fat-soluble vitamins were also rich in iodine, while those poor in fat-soluble vitamins were also relatively poor in iodine It might, therefore, be objected, by those who regard iodine-deficiency as the essential cause of goitre, that the tendency to goitre-production, noted in Fig 13, was an iodine-deficiency effect This was not so, for the tendency to thyroid enlargement was definitely greater in rats fed on incomplete, low protein diets than in those fed on incomplete, high protein diets, although the iodine-ingestion was the same in both, further, the average urinary excretion of iodine by rats fed on incomplete, high and low protein diets was 58 γ per litre in the third experiment and 77 γ per litre in the fourth These figures fall within the normal range of iodine-excretion (56 to 85 γ per litre) by well-fed, non-goitrous stock rats Insufficient absorption of iodine was not, therefore, the essential cause of the tendency to thyroid enlargement, which was noted in rats fed on 'incomplete' low protein diets

The experimental data would appear to admit of a further conclusion, viz, in addition to the *negative factor* of deficiency of fat-soluble vitamins and suitable protein, *an unknown positive factor* was concerned in inducing the tendency to thyroid-enlargement noted in certain individuals It has been seen that the general effect of the experimental diets was to cause the thyroid gland to be *smaller than normal*, what this effect was due to it is not possible to say A less common effect was thyroid-enlargement, and this effect was related to deficiency of fat-soluble vitamins especially in the low protein diets But it was not exhibited by all rats fed on incomplete low protein diets It is necessary, therefore, to postulate the existence, in certain animals, of some goitrogenic agency which tended to counteract the general effect of the experimental diets, and whose operation was favoured by the deficiency of fat-soluble vitamins and suitable protein This agency could not have been another negative dietetic factor, since there was no substance, other than those already discussed (protein, carbohydrate, fat-soluble vitamins and iodine), lacking in the incomplete low protein diets which was not also lacking in the other diets Accordingly, the unknown agency must have been a *positive* factor of some kind or another The only* *positive*, and non-dietetic, goitrogenic agencies so far known are insanitary conditions of life and 'infection' It was not the first of these, since meticulous cleanliness was observed throughout the whole course of the experiments, it may have been the second or some wholly unsuspected agency In the present state of knowledge the important matter is to recognize its existence, for this recognition will surely lead to the discovery of its nature More specific mention of this matter is made by Professor Madhava in his statistical note

* Since this paper was written Webster and his co-workers have shown that cabbage contains a goitrogenic agent

It seems justifiable, therefore, to draw the following conclusions from the data provided by these experiments —

(*a*) The general effect of the experimental diets was to cause the thyroid to be smaller than normal, a less common effect was thyroid enlargement

(*b*) Neither of these effects was the result of high-protein *per se*

(*c*) The tendency to thyroid enlargement was definitely related to deficiency of fat-soluble vitamins in diets containing 20 or less per cent of animal protein It was not related to insufficient absorption of iodine

(*d*) While the tendency to thyroid enlargement was definitely related to *negative* dietetic factors, it seems probable that an unknown *positive* agent was also concerned in causing it

(*e*) This hypothetical positive agent was not insanitary conditions of life, since meticulous cleanliness was maintained throughout the whole course of the experiments, it may have been infection'

Previous experimental work (McCarrison, 1929, 1930*b*, 1930*c* and 1930*d*) has led to conclusions similar to the last three of these and I now feel justified in affirming that in the genesis of at least one type of thyroid enlargement two factors are concerned (*a*) the negative one of vitamin-deficiency, and (*b*) an unknown positive agency, possibly 'infection'

The results of a histological study of these thyroids will form the subject of another paper

Urinary excretion of iodine by rats fed on high protein diets.

The chemical investigations under this heading were carried out by my assistants—Lieut-Col C Newcomb and Dr G Sankaran—to whom I am indebted for the following particulars —

First experiment—In this experiment the intake of iodine was high potassium iodide having been added to the salt-mixture In each of the five groups (0, 20, 40, 60 and 80 per cent protein) the urine of two rats was collected for 16 hours at a time, the animals being fed in different cages during the remaining 8 hours This procedure was adopted in order to prevent contamination of the urine by particles of iodine-rich food finding their way into it The following are the results —

Per cent of animal protein in diet	0	20	40	60	80
Number of estimations of urinary iodine	4	3	3	3	2
Mean body-weight of rats in grammes	139	163	161	153	145
Average volume of urine per rat in 16 hours (c c)	1·62	1·9	2·4	3·68	5·85
Average total iodine in 16 hours urine (γ)	366·6	522·5	700·0	705·4	731·2

These results are shown graphically in Fig 14

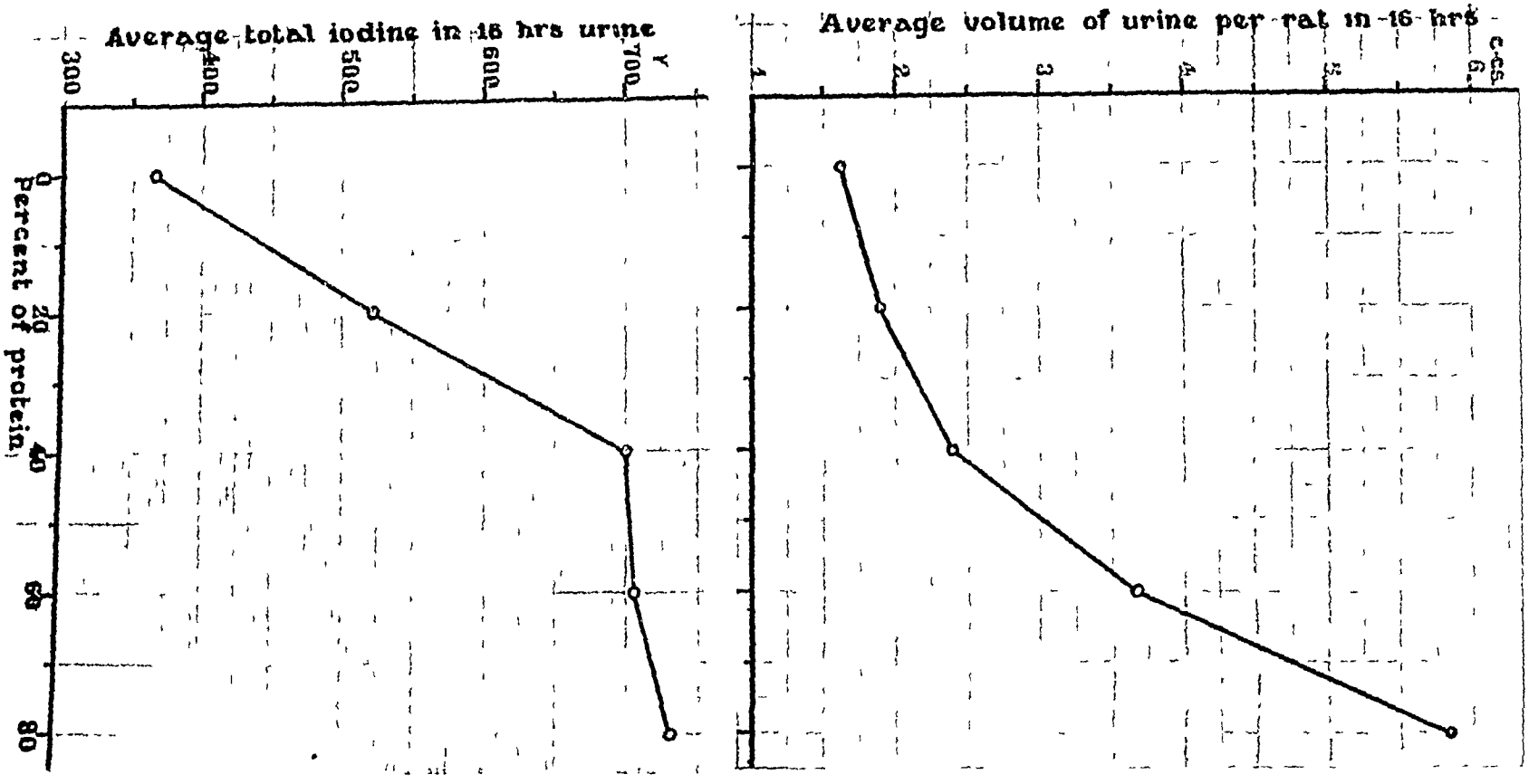

Fig. 14.—Showing the volume of urine and the amount of iodine excreted in the urine in 16 hours by rats in the first experiment

It will be noted that the volume of urine passed in 16 hours rose steadily with increasing amounts of protein in the diet. The total amount of iodine excreted in the urine during the 16 hours also rose as the amount of protein in the diet increased. This increase in iodine-excretion can scarcely be attributed to the iodine-content of the protein, since this was inconsiderable in comparison with the amount contained in the salt-mixture. It suggests rather that the high content of protein in the diet, by increasing the volume of urine excreted, caused a greater excretion of iodine.

Second experiment.—In this experiment 10 rats—two from each group—were kept in metabolism cages for the first 68 days. During the first 43 days of this period they received the experimental diets containing cod-liver oil, during the last 25 days the cod-liver oil was omitted and replaced by the same amount of sesame oil containing radiostoleum (1 drop per 5 c.c. of oil). The amount of food eaten by each rat and the amount of urine passed daily were measured and the amount of iodine excreted in the urine was estimated. The amount of iodine excreted by these rats was insufficient for an estimation in the urine of each rat each day, accordingly, the urine of each animal was collected separately until sufficient had accumulated. In this way six estimations for each rat was made over the 68 days. The results of these observations are set out in Table V.

TABLE V

Showing the average amount of food eaten and average amount of urine passed daily on diets containing various amounts of casein

Per cent casein	Cod-liver oil			Period	Radiostoleum			Period
	Mean body-weight	Food eaten gs.	Urine passed c.c.		Mean body-weight	Food eaten gs.	Urine passed c.c.	
0	129	14·2	5·4	13 days	168	15·0	7·4	25 days
	128	12·3	2·2	„	138	12·9	2·2	„
20	170	13·6	9·0	„	206	14·1	10·6	„
	118	11·1	8·0	„	127	12·1	8·6	
40	148	12·5	7·8	„	184	13·4	9·1	„
	126	11·2	8·0	„	138	11·6	8·9	„
60	148	12·8	11·5	„	176	12·4	13·1	„
	133	11·9	10·3	„	146	11·5	9·5	„
80	101	12·3	12·1	„	119	12·7	15·5	„
	111	12·4	10·6	„	120	11·8	11·0	„

The volumes of urine, calculated per 100 grammes of body-weight, are shown in Fig 15

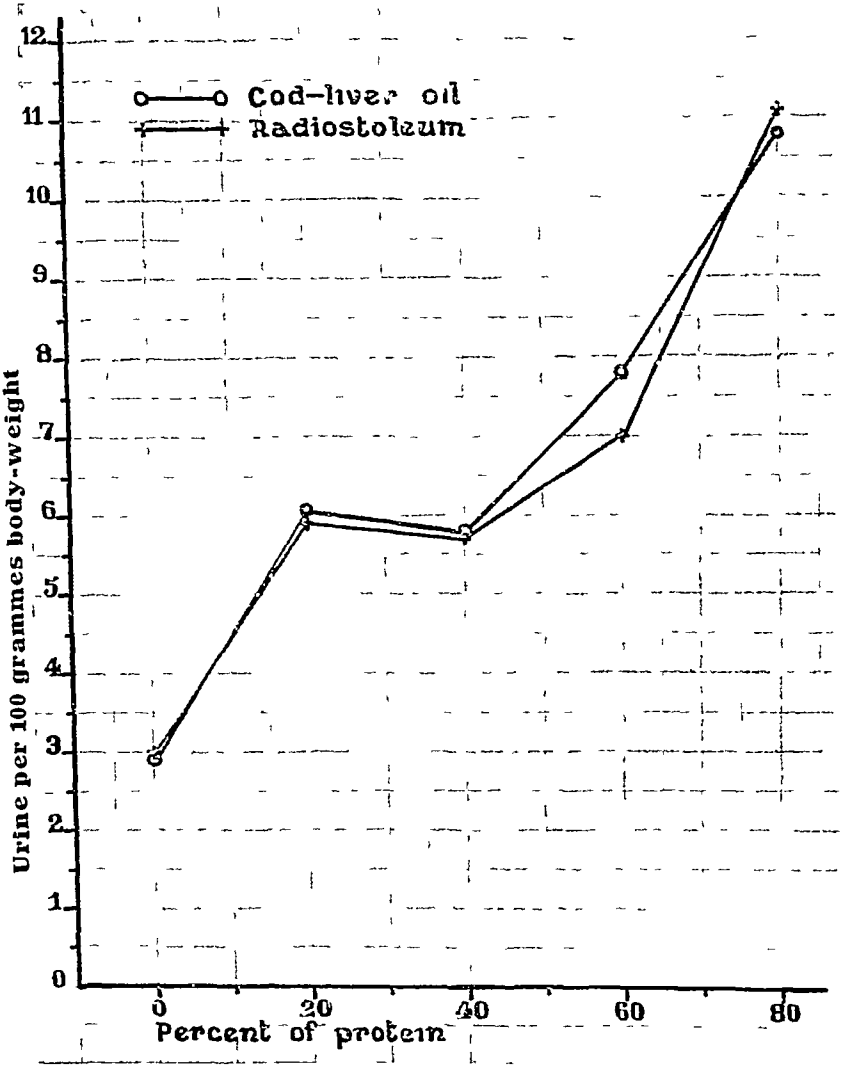

Fig 15—Showing the volume of urine, per 100 grammes of body-weight, passed by rats receiving different amounts of protein, when fat-soluble vitamins were provided as cod-liver oil and as radiostoleum

It will be noted that while the change from cod-liver oil to radiostoleum had no effect on the volume of urine excreted, there was a definite rise in that volume with increasing amounts of casein in the diet

The rats whose diets contained 40 to 80 per cent of casein all had marked albuminuria

The urinary excretion of iodine is shown in Table VI and diagrammatically in Fig 16

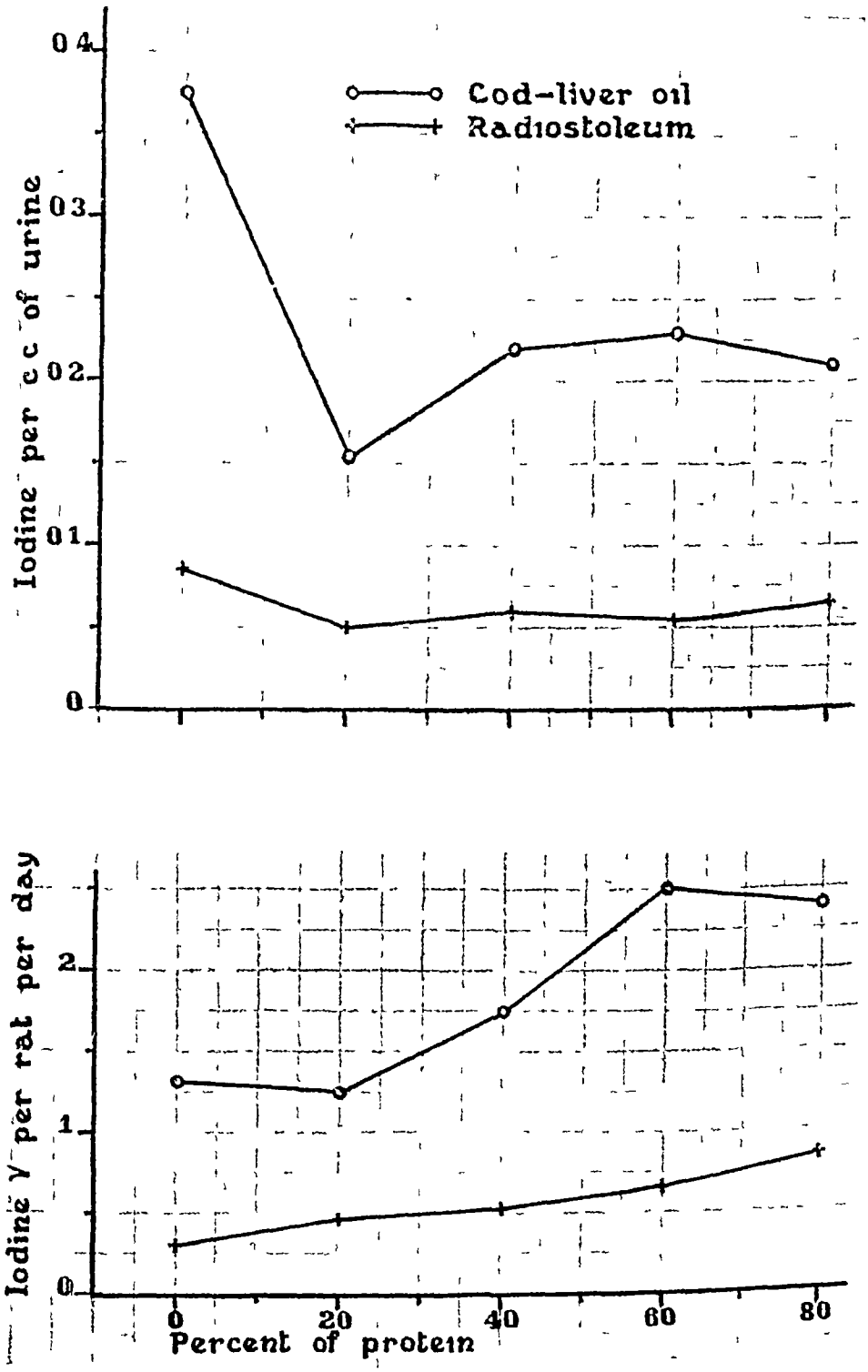

Fig 16—Showing the urinary excretion of iodine by rats receiving different amounts of protein, when fat-soluble vitamins were provided as cod-liver oil and as radiostoleum

TABLE VI

Showing the amount of iodine (in gamma) excreted in the urine

Per cent casein	Cod-liver oil		Means	Radiostoleum		Means
	γ per cc of urine	γ per rat per day		γ per cc of urine	γ per rat per day	
0	0·31	1·67	1·32	0·05	0·37	0·31
	0·44	0·96		0·12	0·26	
20	0·15	1·35	1·28	0·04	0·42	0·47
	0·15	1·20		0·06	0·51	
40	0·21	1·64	1·75	0·06	0·54	0·54
	0·23	1·85		0·06	0·53	
60	0·22	2·53	2·51	0·06	0·78	0·67
	0·24	2·49		0·06	0·57	
80	0·22	2·67	2·40	0·06	0·93	0·85
	0·20	2·13		0·07	0·77	

It will be noticed that there was a marked drop in the urinary iodine on changing from cod-liver oil to radiostoleum solution, due, probably, to the lower iodine-content of the latter. The total amount of iodine (Fig. 16) excreted by these rats rose steadily as the amount of casein in the diet increased, and as the volume of urine excreted increased (Fig. 15). This effect was observed both when the animals were receiving cod-liver oil and when they were receiving radiostoleum. At first sight it might seem that the casein contained more iodine than the other constituents of the diet and that with the increasing proportion of casein in the diet there was a corresponding increase in the amount of iodine ingested, and so in the amount excreted in the urine. It is doubtful, however, whether this is the correct explanation, for if it were, the rise in the total iodine excretion, from 0 to 80 per cent. casein, should have been the same—though at a different level—when the animals were receiving cod-liver oil as when they were receiving radiostoleum. An examination of the levels in Fig. 16 shows that this is not so, even allowing for the admitted approximateness of the iodine-estimations. A more probable explanation seems to be that the casein stimulated iodine-excretion, this stimulation being more marked the higher the level of casein-intake. Further it seems likely that this stimulation is effected by the casein increasing the volume of urine.

Summary and conclusions.

1 A high concentration of animal protein in the diet did not cause thyroid enlargement in growing rats living under conditions of perfect cleanliness Burget's observation (1917) is thus confirmed

2 This result was observed both in animals whose diets were rich in fat-soluble vitamins and in those whose diets were poor in these factors

3 It was observed also in rats whose diets were rich in iodine and whose urinary excretion of iodine was very high as well as in those whose diets were relatively poor in iodine and whose urinary excretion of iodine was relatively low

4 The general effect of the experimental diets was to cause the thyroid gland to be smaller than normal the amount of animal protein, of iodine and of fat-soluble vitamins in the diets did not materially contribute to this result

5 A less common effect of the experimental diets was to cause thyroid enlargement This effect was definitely related to deficiency of fat-soluble vitamins especially in diets having a low content of animal protein It was not related to an insufficient *absorption* of iodine

6 While deficiency of fat-soluble vitamins was the main dietetic factor concerned in causing thyroid enlargement—and without which such enlargement did not occur—it was not the only factor concerned The existence of an unknown *positive* goitrogenic agency is postulated, in addition to the known *negative* one of vitamin-deficiency This positive agency was not insanitation, it may have been 'infection'

7 High protein diets had a marked effect in increasing the volume of urine excreted They gave rise to albuminuria even in animals whose diets contained a sufficiency of fat-soluble vitamins

8 High protein diets appeared to stimulate the excretion of iodine in the urine, and this stimulation seemed to be effected by the protein increasing the volume of urine

9 The ingestion of large amounts of animal protein, in association with an insufficiency of vitamin A, was very prone to cause disease of the urinary, the respiratory and the gastro-intestinal tracts

STATISTICAL NOTE

BY

K B MADHAVA

Having, through the kindness of Colonel McCarrison, had the privilege of access to the statistical material contained in this paper, an attempt is made here first, to construct thyroid curves giving standard weights of thyroids for given body-weights of Coonoor albino rats under three conditions of diet normal, stock diet, 'high' protein diet, and 'low' protein diet, secondly, to examine the differing effects, if any, of 'high' and 'low' protein constituent on the thyroid-weight under the varying conditions of vitamin and iodine-

supply associated with the diets designated 'complete' and 'incomplete' in the text of the paper, and, incidentally, to determine the effect of the radiostoleum introduced in some cases in experiment II

For the purpose of constructing the standard thyroid curves, three sets of data exhibiting the observed thyroid-weights in mg in relation to body-weights corresponding to normal thyroids (N = 107), as well as to those on high and low protein diets (N = 66 and 46, respectively), were available From a preliminary survey of the scatter diagrams of each of these, it was obvious that the relationship was non-linear, and that the equation of regression that should be derived for forensic purposes must involve a logarithmic term, if the relative rapid growth of the thyroid-weights at the lighter body-weights had to be translated into analytical form Nevertheless, in each case, a straight line, a second order parabolic curve, and a logarithmic curve were successively fitted, and from the following standard errors of these estimates confirmatory evidence is obtained in support of the ultimate choice of the logarithmic curves —

	'Normal' thyroid	'High' protein	'Low' protein
Straight line	0 87	1 59	1 46
Parabola	0 95	1 85	0 77
Logarithmic curve	0·84	0 65	0·94

It is satisfying to note also that the equation of relationship for a similar purpose derived by Dr Hatai in the Wistar investigations, and quoted in Dr Donaldson's 'The Rat,' page 218, formula 39, is of the same form The significance of this standard error of estimate is, taking the case of the 'normal' thyroid curve as an illustration, that given a normal distribution of errors, the actual value, if it differs at all from the estimated value, will, in nearly every case, differ with 3 times 0 84 or 2 52 mg , or in 68 cases out of 100, differ with 0 84 mg only, and so on These zones of estimates show the reliability of the estimates themselves, and the limits within which the actual values are likely to fall

Inasmuch as the functional form of relationship between the body-weight and the thyroid-weight in each of the three cases under observation is non-linear, it is necessary to calculate the *index* of correlation, ρ, of a significance precisely similar to the usual coefficient of correlation, r, (when the regression is linear) This is an abstract measure indicating the degree of relationship between the two variables increasing in value up to 1, as the variability of the estimates is materially reduced when the equation of regression is used as a basis for forecasting purposes The values of ρ are 0 980, 0 975 and 0 934 respectively for 'normal' thyroids, 'high' protein thyroids and 'low' protein thyroids, and these high measures afford another criterion in regard to the appropriateness of fitting these observations with logarithmic curves

Finally the equations determined by the method of least squares are as follows —

'Normal' thyroid curve (thyroid-weight in mg) =
0609 (body-weight — 20) + 2 4210 log (Bd wt — 20) — 0 1153

'High' protein diet (thyroid-weight in mg.) =

$$1\ 1320 + 0\ 0017 \left(\frac{\text{Bd wt} - 20}{30}\right) + 10\ 1780 \log \left(\frac{\text{Bd wt} - 20}{30}\right)$$

'Low' protein diet (thyroid-weight in milligrams) =

$$5\ 7614 - 0\ 1634 \left(\frac{\text{Bd wt} - 20}{30}\right) + 11\ 6669 \log \left(\frac{\text{Bd wt} - 20}{30}\right)$$

From these the following standard table (Table A) is constructed giving the weight of the thyroid (in mg) corresponding to given body-weights in each of the three cases, and the same have been graphed together in diagram below —

Diagram

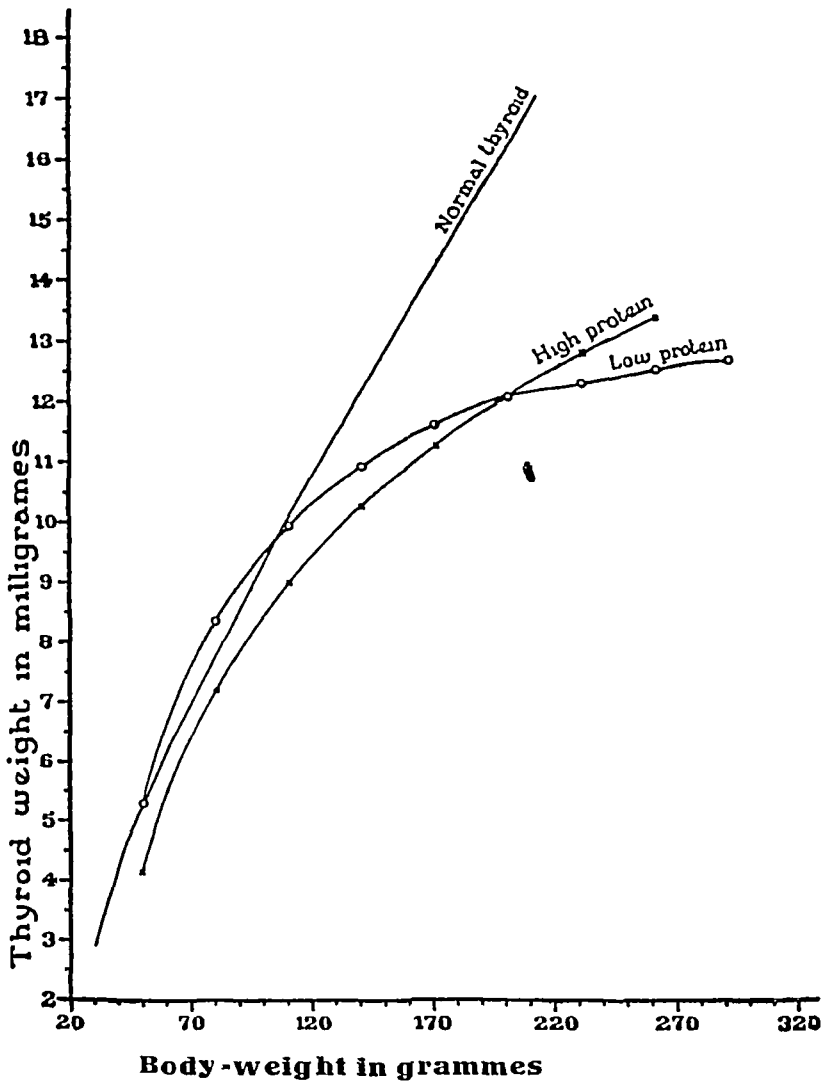

TABLE A

Giving standard weights of thyroids for given body-weights (Coonoor albino rats)

Body-weight in gs	THYROID-WEIGHT IN MG		
	'Normal'	'High' protein diets	'Low' protein diets
30	2 9		
40	4·3		
50	5 3	4 13	5 30
60	6 2		
70	7 0		
80	7 8	7 20	8·35
90	8·6		
100	9·4		
110	10·1	8·99	9·94
120	10·8		
130	11 5		
140	12·2	10·27	10·93
150	12·9		
160	13·6		
170	14 3	11·25	11 60
180	15 0		
190	15 6		
200	16·3	12·06	12·06
210	17 0		
220			
230		12·75	12·28
240			
250			
260		13·34	12 49
270			
280			
290			12·62

It is seen (1) that the standard Coonoor experience of thyroid-weight in relation to body-weight follows in all cases the logarithmic curve, meaning thereby that there is relatively more rapid growth of thyroid-weight at the lighter body-weights than at heavier body-weights,

(2) that as between the differing cases, the 'normal' thyroid is the most steep curve, and the 'low' protein diet curve the most even, the 'high' protein diet curve taking the intermediate position,

(3) that there are differences between each two of these three cases, those between 'normal' and 'high' protein diets being the most conspicuous, and

(4) that in diets with 'low' protein the thyroid weights are higher than under both 'normal' and 'high' protein diets up to a certain body-weight (about 100 gs), but higher than under 'high' protein diets only for body-weights after that and up to about 200 gs, and that ultimately the thyroid-weight is less than under the other two

Moreover the standard table for 'normal' thyroids is more appropriate for use as a basis for comparing the actual thyroid-weight in any observed instance, than the comparison of the thyroid-weights in all observations as so much per 100 grammes of body-weight

With a view to examine the differing effects, if any, of 'high' and 'low' protein constituent on the thyroid-weight under varying conditions of vitamin and iodine-supply, the diets were divided into two types called 'complete' (experiments I and II), and 'incomplete' (experiments III and IV), distinguishing in each case protein composition of 0 and 20 per cent as 'low,' and of 40, 60, and 80 per cent as 'high' The statistical data available may be summarized as follows —

Nature of diet	Symbol	Number of rats	Mean value of the difference in observed thyroid-weights from the normal weight
1 'Complete' 'low'	(AB)	20	—4·265 mg
2 'Complete' 'high'	(Aβ)	27	—3·407 „
3 'Incomplete' 'low'	(αB)	23	—0·357 „
4 'Incomplete' 'high'	(αβ)	33	—0·785 „
5 'Complete'	(A)	47	—3·772 „
6 'Incomplete'	(α)	56	—0·610 „

It is proposed to test whether these samples, or certain pairs among them, belong to the same population, or differ significantly in their means Now since all these samples are small, the necessary analysis has to be made by what is known as 'Student's' method for estimating the significance (P) of

the difference, from the number of degrees of freedom available in the experiments, and (t), the ratio of the observed difference in means to the variance of that difference I have employed throughout this method (described in R A Fisher's 'Statistical Methods for Research Workers,' Chapter V) and the results in this case are as follows —

Samples compared	Degrees of freedom	Difference in means	Value of t	Order of P	Conclusion
I (AB) and ($A\beta$)	45	0 895	1 245	0·2	Not significant
II (aB) and ($a\beta$)	54	0 428	1 049	0·3	Not significant
III (A) and (a)	101	3 162	8·144	Outside the range of Tables— less than 0 01	Significant

Hence (*a*) from I, 'high protein *per se* did not cause enlargement of the thyroid gland when the rats are fed on complete diets',

(*b*) from II 'thyroid-weights of rats fed on incomplete diets also reveal that high protein *per se* did not cause thyroid enlargement',

(*c*) from III, 'as between complete and incomplete diets whether their content of animal protein was high or low, there is a distinct difference,' with of course as may be seen from the results in (5) and (6), that 'in general, the thyroids of rats fed on incomplete diets tended to be larger than those of rats fed on complete diets',

(*d*) also comparing the results in (3) with those in (1), (2) and (4), it is clear that 'there was a definite tendency to goitre-formation in rats fed on incomplete diets which were relatively low in animal protein'

Thus the conclusions of the paper, arrived at on pages 647 and 648 and also stated in summary form, Nos (1) to (5) (page 654) are substantiated

The group (aB) containing 23 rats fed on 'incomplete' diets with 'low' protein, determined above as definitely favouring goitre-production, deserves closer examination There are 8 rats whose thyroid-weights are in *excess* of the normal standard weights to an extent of +2 213 mg on the average, and *15 rats whose thyroid-weights fall short of the standard weights* to an extent of —1 727 mg on the average It is clear that 'the general effect of the experimental diets was to cause the thyroid gland to be smaller than normal', and in the (aB) group we are now examining, the average shortage of all the rats was —0 357, but the 'less common effect,' such as that noticed in the 8 cases above, 'was to cause thyroid enlargement' even in excess of the standard due to normal diet If now the difference between the two subsamples discloses 'significance,' the mere dietetic factors alone considered here do not fully account for this phenomenon, and the heterogeneity must be

ascribed to some other agency not examined here under control As it is, however, the difference between the means is 3 940, the variance 0 512, and hence t is 7 695, there being 21 degrees of freedom, the value of P gets outside the range of tabulated values, is small and definitely less than 0 01 The significance of the difference being thus established, the need for the postulation of 'an unknown goitrogenic agency' arises and is made in the paper The extent to which this agency contributed may be determined by postulating that the value of may be 0 01 (i e , only one value in a hundred) may exceed the outside limits by chance With this value of P, and taking the same value of the variance, viz , 0 512, it is found that a range from —1 81 to +1 09 mg may be allowed for the fluctuations in the influence of the dietetic factor Thus the excess among rats Nos 2840, 2836 and 2838 (being respectively 0 6, 0 7, 0 7) may be ignored, but in the case of rats Nos 2807, 2803 2831, 2811 and 2837 there are additional differences respectively of 0 01, 1 01, 2 31, 3 39 and 3 69 mg

(It will be noted that the large deficiencies in rats Nos 2839, 2802, 2810, 2801, 2804 and 2808 although outside this range are not outside the other dietetic limits determined in this paper)

In the course of experiment II, an incidental attempt was made to seek the effect of the radiostoleum, with the following results —

	Treated with radiostoleum	Others	Together
Number of rats	10	19	29
Mean value of the difference of the thyroid-weight	+1 59	—2·48	1 08

Difference in the mean values between the two diets 4 07, variance 0 803, t 5 071, degrees of freedon 27, hence P is less than 0 01 Hence it *appears* that the difference in the thyroid-weights is significant Nevertheless, among the rats receiving the dose of radiostoleum, four, viz , Nos 2570, 2576, 2591 and 2592, contributed a disproportionately large share in this result Moreover, as the S D is 2 80, even the largest excess amounting to +6 0 (occurring in No 2591) is 2 13 times the S D —that means, that about 3 per cent of normally distributed values will have a deviation of the magnitude noticed here In these circumstances, it is only safe to 'hesitate to conclude that the change from cod-liver oil to radiostoleum during the last 25 days of the experiment was responsible for the larger size of the thyroid gland in the animals receiving radiostoleum'

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STUDIES IN 'PERNICIOUS ANÆMIA' OF PREGNANCY

Part IV

THE PRODUCTION OF PERNICIOUS ANÆMIA (BARTONELLA ANÆMIA) IN INTACT ALBINO RATS BY DEFICIENT FEEDING

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Introduction

THE dietetic survey of various classes of women in Bombay (Wills and Mehta, 1930*a*) including old cases of pernicious anæmia of pregnancy suggested that this disease was closely if not absolutely connected with a relative deficiency of vitamins A and C in the diet In this paper a series of experiments on rats are described in which diets deficient in these vitamins led to the production of a severe anæmia and this anæmia is further shown to be a true Bartonella anæmia The term 'Bartonella anæmia' is used for the anæmia of rats described by Mayer (1921), Lauda (1925) and other workers and without prejudice to the exact nature of the small bodies seen in the red blood cells A review of the literature showed that the general opinion, based largely on work with synthetic diets, is that deficiency of vitamins A and C in the diet does not cause anæmia, but the work of Koessler (1926) and McCarrison (1927) indicates that deficiency of these vitamins has a causal relationship to a certain type of anæmia in rats Koessler produced an anæmia resembling true pernicious anæmia by feeding albino rats on a diet relatively poor in vitamin A McCarrison using diets deficient in vitamins A and C in a series of experiments

designed primarily to produce urinary calculus, reported the occurrence of cases of a severe, and frequently fatal, anæmia, which he described as 'pernicious or pearly anæmia of rats' He most generously placed his records at our disposal, and use has been made of them in this paper

The work, including McCarrison's experiments, was carried out at Coonoor, the stock albino rats of the Nutritional Research Laboratories being used throughout This stock has been raised through many generations at a high altitude (6,000 feet above sea-level) and has become acclimatized to it The entire stock was found to be infested with the rat louse, the necessary vector for *Bartonella anæmia* is, therefore, present Nevertheless this condition has never been observed in the well-fed stock although during the past 8 years an average daily strength of 700 to 800 stock animals has been maintained

As a preliminary to the experiments to be described in this paper a study of the blood was made in this stock with the object of arriving at normal standards An account of this preliminary work has been given in a previous paper (Wills and Mehta, 1930*b*)

Experimental observations.

These observations fall into three categories —

(*a*) The production of *Bartonella anæmia* by diets deficient in vitamins A and C

(*b*) The production of *Bartonella anæmia* by splenectomy

(*c*) The inoculation of *Bartonella*-infested blood into well-fed and into deficiently-fed animals

(*a*) Production of Bartonella anæmia in rats fed on diets deficient in vitamins A and C

Under this heading two series of experiments have to be considered those of McCarrison's referred to above, and our own experiments designed to produce anæmia by dietetic means

First series

Two types of diets were used, the first, diet I, consisting largely of oatmeal, and the second, diets II, III, IV and V, of atta (whole wheat flour) Table I shows the composition of the diets Diet IA was of the same composition as diet I but whole milk was added to it in the proportion of three-quarters of an ounce per rat per day Diet II was the same as diet I, only the oatmeal was replaced by atta In diets III, IV, and V the proportion of atta was higher and the form in which the fat was given varied

The age of the rats at the commencement of the experiments varied from 40 to 70 days, rats of approximately the same age being used in each individual experiment Their weight varied from 45 to 85 gs according to their age group

All these diets, with the exception of IA which is a control diet, are markedly deficient in vitamin A as testified to by the fact that the incidence

of pneumonia, stone in the bladder, cystitis and other infective conditions associated with an A deficiency, was very high among the animals fed on these diets (McCarrison) No source of vitamin C was included in these diets They are judged to contain vitamin B in adequate quantity Vitamin D was provided by exposing the animals daily to the sun The diets are all poor in protein, and this protein being of vegetable origin is of low biological value The results observed in animals fed on diets IV and V show that the nature of the fat can be varied without altering the findings, so long as fats deficient in vitamin A are given The diets were supplied to the animals *ad libitum* Throughout these experiments the animals were kept in screened cages, so that their food and water supply was not contaminated by their excreta

The number of animals fed on the several diets used in this series, and their sex, are shown in Table II together with the incidence of anæmia amongst them One hundred and forty rats were fed on diets of which the basis was oatmeal, amongst these there were 19 cases of anæmia, or an incidence of 13 5 per cent One hundred and thirteen rats were fed on diets of which the basis was atta, amongst these the incidence of anæmia was 12 4 per cent, practically the same as that amongst animals fed on the oatmeal diets It is to be noted that no case of anæmia occurred amongst 18 animals fed on the oatmeal diet to which whole milk was added The disease appears to have been completely prevented by this addition It is to be regretted that both in this series and in the Mohammedan diet experiment to be reported later, there were not more direct controls The incidence of the disease was higher

TABLE I

Diets First series

	DIETS PER CENT				
	I	II	III	IV	V
Oatmeal	53				
Atta		53	78	90	90
Linseed meal	20	20	20		
Cornflour	25	25			
Linseed oil				8	
Gingelly oil					8
Sodium chloride	1	1	1	1	1
Calcium phosphate	1	1	1	1	1
TOTAL	100	100	100	100	100

in females, twice as many females as males dying of it Amongst the 30 females in the first oatmeal group 7 became pregnant of these 7 animals 5 died of anæmia

In this series other milder cases of anæmia no doubt occurred, but were not specially noted as the question of anæmia was not under consideration and no blood counts were made The true incidence of the disease was probably, therefore, considerably higher than that shown in Table II

The diagnosis of 'Bartonella anæmia' in this series was made after we had examined blood slides taken from certain of these animals but not previously studied

As the blood picture, the clinical picture, and the post-mortem findings are the same in both series a detailed description of these will be deferred till after the second series has been discussed

TABLE II

Incidence of Bartonella anæmia in intact animals on oatmeal and atta diets

	Diet	Number of animals	Males	Females		Deaths from anæmia			Death-rate Per cent
				Total	Pregnant	Total	Males	Females	
I	Oatmeal	72	30	42	7	13	2	11	18·0
	„	50	20	30		3	?	?	6·0
	„	18	9	9		3	3	0	16·6
	TOTAL	140	59	81	7	19	5	11	13·5
IA	Oatmeal plus milk	18	9	9		0	0	0	0·0
II	Atta	50	20	30		3		2	6·0
III	„	21	10	11		6	3	3	28·5
IV	„	21	10	11		1	1		4·7
V	„	21	8	13		4		4	19·0
	TOTAL	113	48	63		14	4	9	12·4

Second series

In this series of experiments 6 diets were used Of these the first and second were natural diets based on those in common use by women suffering from anæmia of pregnancy, the remaining 4 were synthetic diets The first resembled that eaten by Mohammedan women, it will be referred to hereafter

as 'the Mohammedan diet' The second resembled that eaten by Hindu women, it will similarly be referred to as 'the Hindu diet' The four synthetic diets were used for a dual purpose to determine whether or not anæmia could be produced in rats by means of synthetic diets deficient in certain vitamins, and to act as 'controls' to 'the Mohammedan' and 'the Hindu' diets The composition of these two diets is given in Table III

The average age of the rats at the beginning of these experiments was 40 days and their average weight approximately 45 gs Equal numbers of males and females were used

TABLE III

Second series Natural diets

	Mohammedan *diet* Per cent	Hindu diet Per cent
Polished rice	30	50
White bread	40	
Atta	10	27
Bajree		15
Dhal		3
Ghee	10	
Gingelly oil		4
Pumpkin (white)	5	1
Meat	5	
Salt	Trace	Trace
TOTAL	100	100

(1) '*The Mohammedan diet*'—The rice, vegetable and meat were cooked together with a little salt in the manner customary amongst Mohammedans The atta was made into *chapattis* with part of the ghee All the ingredients were then intimately mixed and fed to the animals in excess Water was supplied *ad libitum* The ghee used in the preparation of this diet was purchased in the local market, repeated colour tests showed it to be devoid of vitamin A The rice and atta were obtained in Bombay from the sources used by our patients This diet was not only relatively deficient in vitamins A and C but was also low in vitamin B and had the relatively large amount of fat so characteristic of the diets used by the poorer Mohammedans in Bombay (Wills and Mehta, 1930*a*) Its deficiency in vitamin D was made good by exposure of the animals fed upon it to the sun

On this diet the animals grew very little, the average gain was only 60 grammes in 87 days (from 49 to 109 gs), suffered from loss of hair, especially on the shoulders, and from a high incidence of infective conditions (pneumonia, abscesses, septic eyes) which can be regarded as evidence that the diet was low in vitamins B and A

(2) *'The Hindu diet'*—The atta, bajree (pearl millet) and oil were made into *chapattis*, the remaining constituents of the diet were boiled together After cooking, the food was intimately mixed and given to the animals in excess Water was supplied *ad libitum*

This diet is a purely vegetarian one, well calloried, and containing good grains which supply a better quantity of vitamin B But the absence of animal fats and the low vegetable content make the supply of vitamins A and C relatively low Its deficiency in vitamin D was met by exposure of the animals fed upon it to the sun

A control to this diet was provided by increasing its scanty supply of vitamins A and C by the addition of cod-liver oil (1 per cent) and tomato-juice (2 c c), 'the Hindu diet' so modified will be referred to hereafter as the 'control Hindu diet' On both these Hindu diets the animals grew better than those on the Mohammedan diet (average figures useless as so many animals became pregnant), and did not loose their hair but the experimental group suffered severely from infective conditions (3 pneumonia, 3 septic eyes, 4 septic tails) which indicates that the supply of vitamin B was fairly adequate, but that of vitamin A was low

(3) *The synthetic diets*—The composition of these is given in Table IV Of these diets, No 1 was deficient in both vitamins A and C, No 2 was relatively deficient in vitamin A, No 3 only in vitamin C, while No 4 was complete The animals were exposed to sunlight daily

The experiment was stopped at the 11th week

The rats on the complete synthetic diet grew and remained perfectly well throughout the experiment, and many of the females were pregnant at its conclusion However the fact that the rate of growth fell from 11 gs per week during the first month to 8 5 at the 11th week suggests that though the vitamin B-content of the diet was adequate it was not maximal On diet No 1 (deficient in both vitamins A and C) the rate of growth was less from the beginning, and fell rapidly after the third week to drop at death to levels well below the maximum values On the other two diets the rats grew normally for the first three weeks and after that in both cases the rate of growth flattened but more markedly in those rats on a relatively A poor diet Table V shows the results of these feeding experiments as regards anæmia The diagnosis of anæmia was made on the clinical signs such as extreme pallor, and confirmed by the characteristic blood picture (*vide infra*) in the affected animals and by the presence in the red cells of Bartonella bodies

It will be noted that of the six diets used in this series of experiments, only the Mohammedan, and the synthetic without vitamins A and C caused

anæmia In these two diets the common dietary fault was the deficiency of vitamins A and C 'The Mohammedan diet' was also faulty in being too low

TABLE IV

The synthetic diets

Ingredients	DIETS PER CENT			
	I	II	III	IV
Purified starch	60	60	60	60
Casein (vitamin free B D H)	20	20	20	20
Olive oil* (Lucca)	15	15	15	15
Salt mixture (McCollum's)	5	5	5	5
Yeast (Harris's concentrated powder)	0·1 gramme per rat per day			
Cod-liver oil (per cent)	0	0	2	2
Orange juice per animal (in c c)	0	2	0	2
Water	*ad libitum*			

**It is possible that this contained some vitamin A*

TABLE V

Diets	NUMBER OF ANIMALS			NUMBER OF CASES OF ANÆMIA			Morbidity rate Per cent
	Total	Males	Females	Total	Males	Females	
Mahommedan	36	18	18	7	2	5	16·6
Hindu	30	10	20	0	0	0	0·0
Hindu Control	15	5	10	0	0	0	0·0
Synthetic I	6	3	3	2	1	1	33·0
Synthetic II	12	6	6	0	0	0	0·0
Synthetic III	12	6	6	0	0	0	0·0
Synthetic IV	12	6	6	0	0	0	0·0

in vitamin B, animal protein and salts whereas in the synthetic diet (No I) the vitamin B, animal protein (casein) and salts were present in adequate

amounts 'The Hindu diet' is also relatively low in vitamins A and C and animal protein is absent, whereas vitamin B is present in larger amounts owing to the better cereals eaten, but though a mild anæmia occurred in all the pregnant animals there were no cases of Bartonella anæmia The synthetic diets on which no case of Bartonella anæmia occurred were either complete or deficient in only one vitamin

Description of the severe dietetic (Bartonella) anæmia

1 *Onset*—The onset was apparently very sudden as, though the animals were examined daily, signs or symptoms were never observed till the blood count had already fallen very markedly, and the animal, which on the previous day had appeared normal, was obviously anæmic and ill On the Mohammedan diet cases occurred as early as the 44th day of experimentation (animals 78 days old), but cases could occur as late as the 104th day (on atta diet) In the authors' series all the cases developed within a period of 10 days, which suggests a small epidemic, but in McCarrison's earlier series cases were recorded over a period of six months, though here again small groups of cases frequently occurred Sporadic cases on other similar diets were also recorded

2 *Clinical features*—The most striking feature of these cases was the extreme pallor, the eyes were pale pink instead of their normal brilliant ruby red colour, and the ears and feet were very bloodless The animals were obviously ill, inactive and disinclined to eat Hæmoglobinuria was present in the severe cases In the first series only, the animals that died from the anæmia are under consideration The pregnant females generally died undelivered In the second series, with the exception of one female all recovered spontaneously

3 *Blood picture*—The blood findings in a typical case that recovered are given in Table VI The red cell count may fall from 10 millions to 1 35 millions and the hæmoglobin from 96 per cent (13 2 gs) to 28 per cent (4 9 gs) This decrease in the red cell count is associated with a marked variability in the size of the red cells, the average diameter increases from 5 9 μ to 8 2 μ and in addition a large number of very large cells are present Plate XLIV, figs 1, 2 and 3 are reproductions of photographs of blood films from normal and anæmic rats the magnification is the same in all three Chart 1 gives the Price-Jones curves of normal and anæmic rats and shows this change in the size of the red cells very clearly This increase in size is associated with an increased colour index as in true pernicious anæmia so that it is probable that the cells are carrying their maximum load of hæmoglobin The blood films show marked polychromatophilia and frequent Howell-Jolly bodies in the red cells As the anæmia develops numerous normoblasts appear in the blood stream and as healing commences a large number of reticulocytes are to be found But the finding that clinches the diagnosis of Bartonella or pernicious anæmia of rats is the presence of the characteristic rod-like Barton bodies

TABLE VI

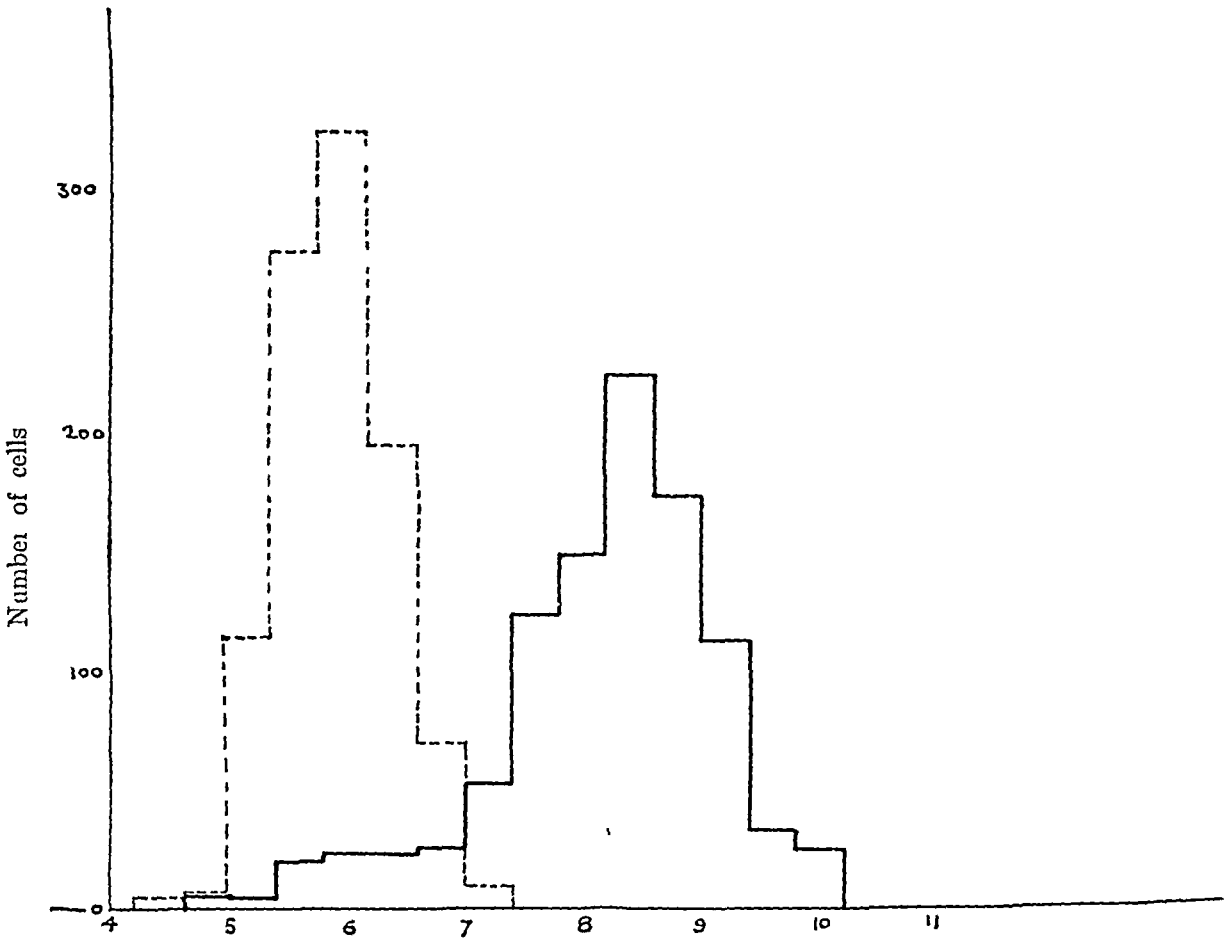
Blood changes in Bartonella anæmia

Number	Date	R B C per c.mm in millions	Hb per cent, 100 per cent = 13·80 gs Hb per cent	*Colour index	W B C per c.mm	Differential white count per cent: Polymorphonuclears Per cent	Eosinophiles Per cent	Lymphocytes Per cent	Large lymphocytes Per cent	Myelocytes Per cent	Blood picture: Anisocytosis	Poikilocytosis	Polychromatophilia	Howell-Jolly bodies	Megaloblasts: Per 100 W.B.C counted	Normoblasts	Average diameter red cells in μ	Reticulocytes Per cent	Organisms (Bartonella)
Intact rat female on A-C-deficient diet																			
W 40/1	17 S 29	10 050	96	0 90	6,200	25		63	12		−	−	−	−			5 9	2 0	−
	2 9 29	1 600	21 5	1 33	9 000	18		78	1		+++	−	+++	+	2	54	6 3	Very few	−
	5 9 29	1 350	28	2 05	8,600	12		84	3	1	+++	−	+++	+		42	7 6	0 1	+
	7 9 29	1 420	32	2 23	3,800	23		70	6	1	+++	−	+++	+		46	8 2	15	+
	10 9 29	3 400	55 5	1 61	10 000	18		68	14		++	−	++	+		24	7 7	51	−
	19 9 29	5 690	78	1 36	6 400	34		57	9		±	−	−	−			6 6		−
Splenectomized rat operation on 10-9-29																			
S 1	10 9 29	10 150	113	1 09	10 100	30		62	8		−	−	−	−			5 65	0 1	−
	13 9-29	8 970	92	1 01	20 000	56		32	12		−	−	−	−			5 75	5 0	−
	15 9 29	6 866	69	0 99	41,000	61		32	7		±	−	±	−			6 10	0 0	+
	17 9 29	2 170	25	1 13	71,000	66	5	23	6		+++	−	+++	+	2	50	6 50	0 0	++
	19 9 29	2 400	35	1 44	50,700	14	5	62	19		++	−	++	+	1	57	7 75	4 0	++
	21 9-29	2 050	40	1 92	41,800	8	2	70	18	2	++	−	++	+		42	8 20	65 0	+
	24 9 29	4 100	56	1 35							++	−	++	+			8 30	2 0	−
	2 10 29	6 710	89	1 31	17,400	38		52	10		+	−	±	+			7 20	3 0	−
	7-10 29	9 200	109	1 09	14,800	39		46	15		±	−	−	−			7 10	Not done	−

*14 grammes hæmoglobin per cent = 100 per cent hæmoglobin, and 10·0 million red cells per c.mm = 100 per cent red cells

in the red cells at the height of the anæmia These organisms stain well with Giemsa and show as reddish blue rods, they are absolutely characteristic of the disease (Plate XLIV, fig 4)

CHART 1

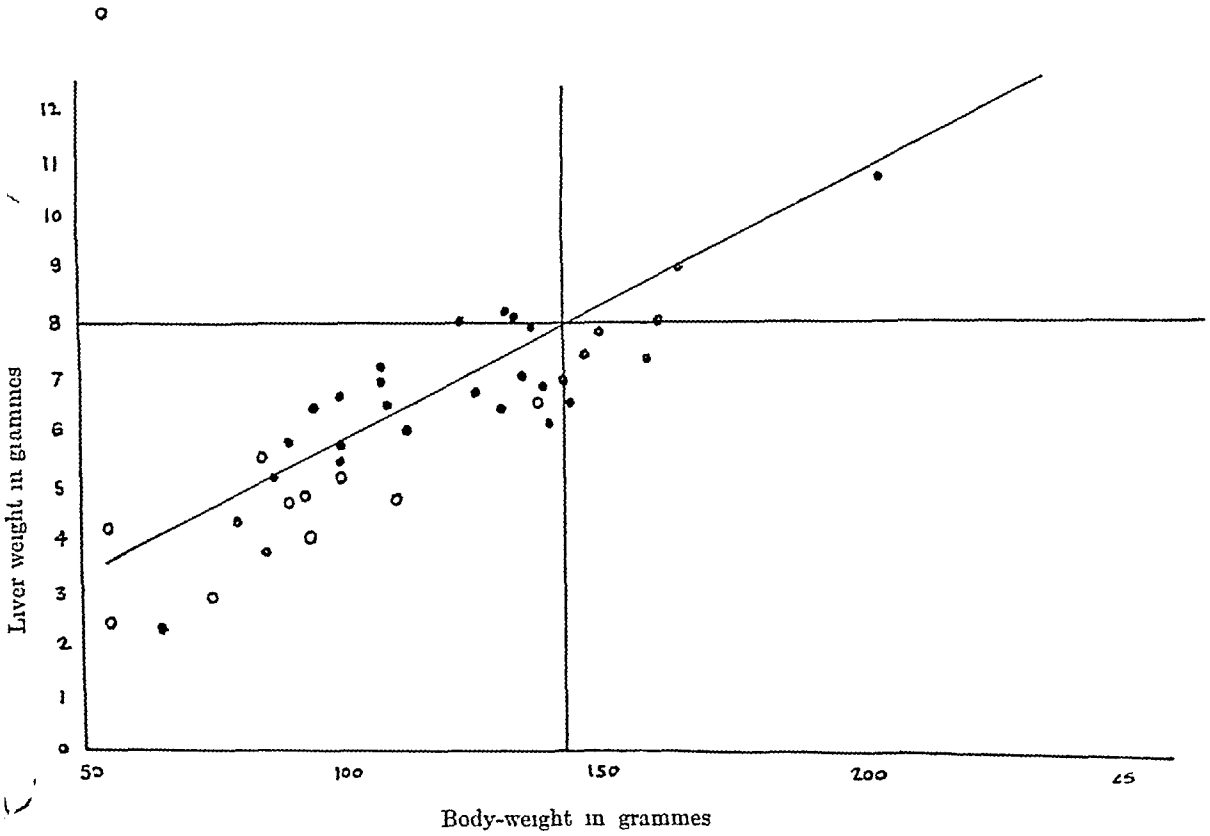


The white cell count decreases as the anæmia develops, the lowest value in the case quoted being 3,800 per c mm , a very low value for an adult rat There is no increase in the polymorphonuclear elements At the height of the anæmia a few myelocytes appear in the blood stream

4 *Post-mortem findings* —The post-mortem findings in these animals are very characteristic There is extreme pallor of all organs, especially of the pancreas which is always dead white The liver is strikingly altered fatty changes are marked and give it a pale mottled appearance which is very

characteristic of the severe cases On inspection it gives the impression of being enlarged but when the weight is plotted against body-weights it is found to lie within normal limits (Chart 2) The spleen is sometimes definitely enlarged (Chart 3) and has been described as a 'chestnut spleen' If there has

CHART 2



Liver weights in grammes charted against body-weights in grammes

Regression line for observations on McCarrison's normal rats

• Liver weights—Splenectomized anæmic rats

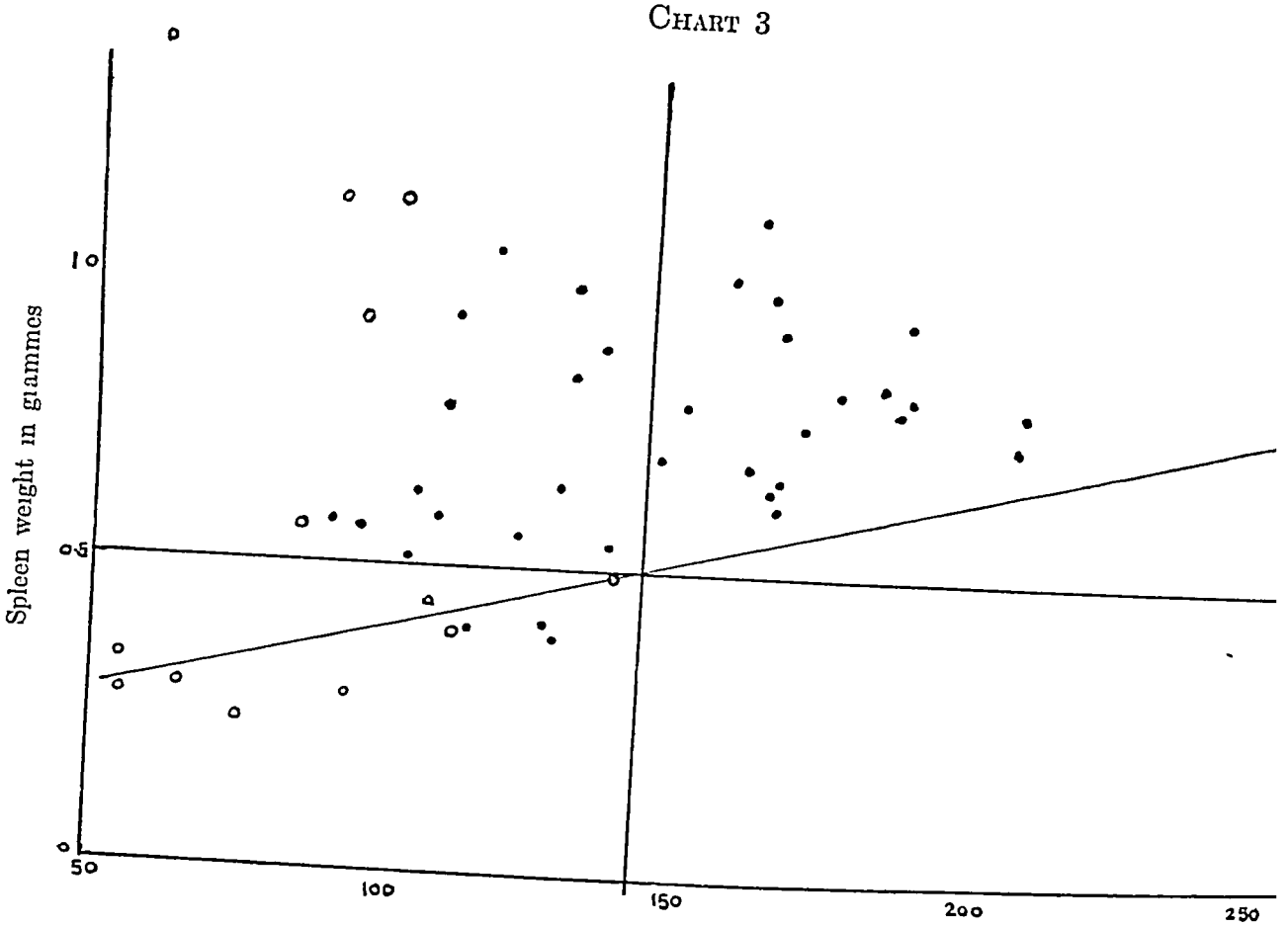
○ „ „ —Intact anæmic rats

Mean body-weight 143 δ_1 35 3

Mean liver weight 7 94 δ_2 1 94

been hæmoglobinuria the kidneys are enlarged and very dark in colour from retained pigment and the bladder is frequently distended with blood-stained urine If there has been no hæmoglobinuria the kidneys and bladder appear normal The bone-marrow shows no constant macroscopic changes

CHART 3



Spleen weights in grammes against body-weights in grammes

Regression line for observations on McCarrison's normal rats

pleen weights—Spleen removed at operation
" " —Intact anæmic rats

Mean body-weight 143 δ_1 35·3
Mean spleen weights 0·52 δ_4 0·17

(*b*) BARTONELLA ANÆMIA FOLLOWING SPLENECTOMY

In order to confirm the findings in the dietetic experiments and to secure material for inoculation experiments a number of rats were splenectomized A brief review of the literature of this subject is given below

LITERATURE

This review of the literature of Bartonella anæmia must necessarily be incomplete as all the journals are not available but the main lines of work are reported Sorge (1928) gives a good review of our knowledge of this condition up to 1928 It is a severe anæmia that occurs in most strains of rats after splenectomy, it is of sudden onset, frequently fatal in a few days and generally associated with profuse hæmoglobinuria The death rate is frequently high but mild or abortive cases (Lauda, 1925) occur and relapses are not uncommon and may prove fatal

It is generally agreed that the anæmia is an infective one associated with the presence of rod-like bodies (*Bartonella muris ratti*) in the red blood cells, though the exact nature of these is still in dispute The anæmia does not occur in intact animals but only after splenectomy, and Ford (1928) states that he has only once seen a Bartonella organism in the blood of whole albino rats though these have been reported in wild rats and other rodents though not associated with an anæmia Sorge (1928) also states that he has never seen either organism or symptoms of anæmia in intact albino animals There are non-infected strains in which removal of the spleen is not followed by anæmia, animals from such can be infected either by contact with infected stock or by the inoculation, intraperitoneally, intravenously or subcutaneously, of blood or emulsion of liver substance from infected animals Animals so infected will only develop anæmia after splenectomy, though infection may occur either before or after the operation (Sorge, 1928, Cannon and McClelland, 1929) Lauda claims to have produced the disease in intact animals by simultaneous injection of infected material by intravenous, subcutaneous, intraperitoneal and intrahepatic routes Ford (1928) states that young rats under 30 days old and under 20–30 gs in weight can be infected by the inoculation of infected material and proposes to use such animals to demonstrate the presence of the virus

Experiments by Mayer (1928), Sorge (1928), Cannon (1929), and others have shown that the infection is spread by the rat louse and possibly by other ecto-parasites

The nature of the Bartonella bodies in the red blood corpuscles and the question of the infective nature of the disease cannot be decided finally till the organism, if such it be, has been isolated and cultured For the following reasons, however, the weight of the evidence would seem to be in favour of the Bartonella bodies either being the infective agent or of some virus associated with them in or on the red cells

(1) The disease is never seen without the Bartonella bodies appearing in the red cells and the severity of the anæmia in splenectomized rats is directly proportional to the number of cells infected with these bodies

(2) The infection can be carried through several generations by animal passage (Ford, 1928)

(3) The inoculation of washed red corpuscles from infected animals leads to the development of the anæmia in suitable animals (Ford, 1928)

(4) The fact that splenectomy in non-infected animals does not cause anæmia (Haam, Lauda and Sorge, 1927, Mayer, 1928, and Cannon, 1929) though this can be produced by exposing the animals to infected stock or by the inoculation of blood from such Bayon (1928), Mayer (1921), and Noguchi (1928) claim to have grown the organism in a few instances but others (Ford, 1928) have failed to confirm this

The blood picture and post-mortem findings are well described by Sorge (1928) The blood picture is that of macrocytic anæmia The increase in size of the cells is associated with a raised colour-index The films show marked anisocytosis and polychromatophilia and very little poikilocytosis There are normoblasts and immature cells with numerous Howell-Jolly bodies and a high percentage of reticulocytes In the disease as it appears in splenectomized rats there is a great increase in the number of white cells, especially the neutrophile polymorphonuclear leucocyte, and there may be phagocytosis of erythrocytes by large mononuclear cells The parasites, which stain well with Giemsa and are Gram negative, appear in large numbers in the red cells and in severe cases nearly every red corpuscle may contain the rod-like bodies, and their numbers are directly related to the severity of the anæmia The red count may fall as low as 1 million Ford (1928) reports that in young rats there is not an enormous increase in the granular leucocytes, which increase he thinks is due to the splenectomy and not to the infection

Post-mortem the most characteristic findings according to Sorge, are the extreme paleness of all the tissues and increase in size and mottling of the liver The kidneys are deep red in colour and may be a little enlarged Histologically the most characteristic changes are in the reticulo-endothelial cells and these show phagocytosis of erythrocytes with intracellular

deposits of drops of hæmoglobin and granules of iron pigment and fatty degeneration these changes are most marked in Kupfer's cells The changes in the kidneys are secondary to the hæmoglobinuria

Sorge (1928) reports the cure of the condition by organic arsenic preparations, especially neosalvarsan and Mayer (1928) uses this sterilizing effect of neosalvarsan to secure a strain of non-infected rats

Bartonella infections occur in other rodents and in the dog (Kikuth, 1929), but the anæmia only develops after splenectomy In man the allied organism *Bartonella bacilliformis* is the cause of Oroya fever and Verruga Peruviana the former being a disease associated with a very severe hæmolytic anæmia, with a blood picture, according to Stitt (1929), closely resembling that of pernicious anæmia of rats

TABLE VII

Results of splenectomy in 65 rats

Group Post-operative treatment	Total number	Rats developed anæmia		Rats remained well	
		Number	Percentage	Number	Percentage
Untreated	25	24	96	1	4
Fed liver extract	17	14	82	3	18
Fed fresh spleen	12	10	83	2	17
Fed vitamin A	11	11	100		

Results of splenectomy

Sixty-six rats, all males, were splenectomized by Colonel McCarrison, the anæsthetic (ether) being given by the senior author These animals were representative samples of the breeding and experimental rats and were taken from all three animal houses In the present experiment each group of animals was made up from corresponding samples so that though each individual group contained different types of animals, all the groups had the same variables and so were comparable The operations* was done under open ether One animal died 12 hours after the operation from hæmorrhage from the pancreas, it also had pneumonia The others survived and seemed very little the worse for the operation till symptoms of anæmia developed several days later Post-operatively the animals were kept in separate cages and fed on stock diet with the addition of unlimited whole milk Twenty-five rats had no further treatment 17 were fed liver extract in doses equivalent to 6 grammes fresh liver daily 12 were fed fresh spleen approximately 1 gramme daily and 11 were treated with Radiostoleum (vitamin A concentrate) in 3 drops doses twice daily

From Table VII which gives the results of operation, it is clear that the whole of the stock was liable to develop anæmia after splenectomy Blood

* *See* Appendix for operative method

films taken from these animals at the height of the anæmia all showed the presence of numerous Bartonella bodies in the red cells, the number of organisms being directly proportional to the severity of the anæmia, in other words, the anæmia was a Bartonella anæmia The fact that a few animals fed on liver extract and spleen pulp and only one in the control group did not develop the disease does not indicate that liver and spleen therapy protect from this complaint, as the difference between the groups is not significant Vedder (1928) has also reported that liver and spleen extracts have no protective action in this anæmia in splenectomized rats Vitamin A also failed to protect the animals Many of the animals recovered spontaneously but the death-rate could not be ascertained as many were killed at the height of the anæmia for experimental purposes

(*c*) Inoculation experiments

The following experiments were designed with a view to producing an attack in susceptible animals by inoculation of infected blood, which was obtained by withdrawing heart blood from splenectomized animals at the height of the anæmia, when the red cells were heavily infected with Bartonella organisms

1 Ford's (1928) work was repeated and 30 days old rats inoculated but no anæmia resulted and no organisms were found in the films

2 Ten healthy stock males were inoculated intravenously with quantities varying from 0 3 to 1 c c of infected blood No animal became anæmic and no organisms were seen in blood films, in fact the animals appeared absolutely unaffected by the injection

3 Four normal female animals were inoculated intraperitoneally with 0 5 c c infected blood, none became anæmic and the animals remained in perfect health Table VIII, 1, shows the findings in one animal

4 Four normal pregnant females were inoculated intraperitoneally with 0 5 c c infected blood One animal was killed accidentally on second day of the experiment, another showed no sign of anæmia and remained well and gave birth to a healthy litter that survived The other two animals showed a slight reduction in both the red cell count and the hæmoglobin value associated with a slight anisocytosis and some polychromatophilia No organisms were seen in the films The animals appeared perfectly well Table VIII, 2, shows blood changes in one of the two rats that became slightly anæmic

5 Four pregnant rats fed on the 'Hindu diet' were inoculated intraperitoneally with 0 5 c c of infected blood Two of these animals were on 'the control Hindu diet,' that is were receiving cod-liver oil and tomato juice in addition to the diet One of these unfortunately died from prolapse of the uterus a few days after the inoculation, before any very marked changes had occurred in the blood The other developed a mild anæmia (Table VIII, 3*a*), unassociated with any changes in the blood picture The animal appeared perfectly well The two other animals were on the uncorrected Hindu diet

TABLE VIII

Blood findings after inoculation of infected blood into female animals

Group	Class	Volume blood inoculated in c c	Days from inoculation	R B C per c mm	Hb Per cent	Colour index	W B C per c mm	Polymorphonuclears Per cent	Lymphocytes Per cent	Large lymphocytes Per cent	Anisocytosis	Polychromatophilia	Howell-Jolly bodies
1	Controls, females non-pregnant	0·5	Before 1	9,183,000	101	1 09	15,800	25	70	5	—	—	—
			After 7	9,110,000	105	1 14	14,600	17	69	14	—	—	—
2	Controls, females pregnant	0·5	Before 1	8,500,000	92	1 06	10,200	20	75	5	—	—	—
			After 1	7,700,000	86	1 10	8,200	48	47	5	—	—	—
			„ 2 *	7,280,000	77	0 90	16,000	30	63	7	+	±	—
			„ 3 †	7,480,000	80	1 06	24,000	44	46	10	—	—	—
			„ 6	8,960,000	93	1·02	20,800	37	15	18	—	—	—
3	*a* Pregnant females on Bombay Hindu diet corrected	0·5	Before 1	8,230,000			13,000	23	71	6	±	—	—
			After 4 ‡	7,750,000	86	1 10	8,800	40	43	17	—	—	—
			„ 8	8,080,000	99	1 21	11,600	7	74	19	—	—	—
3	*b* Pregnant females on Bombay Hindu diet uncorrected	0·5	Before 1	9,020,000	95	1 04	13,100	10	82	8	—	—	—
			After 2	7,600,000	90	1 17	12,100	35	56	17	+	—	—
			„ 3	4,590,000	60	1 29	17,200	24	67	9	+	+	+
			„ 4	4,480,000	53	1 16	11,000	36	52	12	++	+	+
			„ 5	5,180,000	?	?	13,600	27	53	20	++	+	+
			„ 6 §	4,766,000	64	1 32	17,500	57	36	7	++	+	+
			„ 7	5,930,000	?	?	10,600	34	58	8	++	+	++
			„ 14	8,170,000	99	1 19	19,600	47	44	9	±	±	—

* Delivered † Young died ‡ Delivered young alive § Delivered 7 young alive

These two animals differed from all the others inoculated in that they became severely anæmic and clinically appeared ill, whereas all the others showed at the most a very slight anæmia and seemed otherwise entirely unaffected by the inoculation The changes that occurred in the blood counts and picture of the two animals were very similar, so only one will be considered in detail (Table VIII, 3*b*) Before the inoculation the red cell count was 9 00 million per c mm , 4 days after the inoculation it had fallen to half that value whereas the hæmoglobin had only fallen from 95 to 53 per cent, so that the colour-index had risen from 1 04, a normal value, to 1 29 which is an increased value This increase in the colour-index was associated with the appearance of numerous large, frequently polychromatophilic red cells in the blood, so that the slide showed marked anisocytosis and polychromatophilia Howell-Jolly bodies were also frequent After delivery there was a fairly rapid recovery with a return to a normal blood picture The white cells count before and throughout the experiment was raised and except for the usual increase in polymorphonuclear cells after delivery the ratios of the different cell elements fell within normal limits Though the slides were searched again and again no Bartonella organisms were discovered

All attempts to culture the 'organism' failed

Discussion.

The present series of experiments have demonstrated that it is possible by dietetic means to produce a severe megalocytic anæmia in rats, especially in female animals, and further to show that this anæmia is a Bartonella anæmia occurring in intact animals Two of the experimental diets were natural diets based on those in common use among the women who suffer from pernicious anæmia of pregnancy in Bombay These differ in their composition and in their effect on the animal The first was the so-called Mohammedan diet This diet is not only low in vitamins A and C but also high in fat and relatively deficient in B compared to the Hindu diet, as atta is partially replaced by white bread, and bajree and dhal are not included in the diet On this Mohammedan diet severe cases of Bartonella anæmia occurred in female animals On the 'Hindu diet' which was not only less deficient in vitamin A but contained less fat and also a more adequate supply of vitamin B there were no spontaneous cases of Bartonella anæmia However, pregnant animals on this diet, when inoculated intraperitoneally with Bartonella infected blood, became extremely anæmic This fact is significant as other pregnant animals, whether normally fed or receiving the supplemented Hindu diet, failed to develop this severe anæmia after inoculation.

The other natural diets (McCarrison's stone-producing diets) that produced this anæmia were not based on Bombay diets but shared the same vitamin deficiency, i e, a partial deficiency of A and C That the causal factor is either this dual deficiency or some other related to it, possibly a specific anti-anæmia factor as found in liver, rather than a general deficiency of vitamins

A, B, and C, as found in the Mohammedan diet, is suggested by the results of these experiments of McCarrison and the experiments with synthetic diets The vitamin B-content of all these diets was judged to be adequate, but this did not prevent cases of anæmia from occurring in rats fed on those that were low in vitamins A and C

It has been shown that this dietetically-produced anæmia is a Bartonella anæmia as it was possible to demonstrate the organism in the red blood cells Further splenectomy experiments showed that the whole of the Coonoor stock was liable to develop this anæmia under suitable conditions and yet, out of all the animals undergoing experimentation both while the present series were under investigation and during the earlier work, only those on diets low in vitamins A and C developed this anæmia Other animals in close contact were deficiently-fed and below par but these were immune

It seems safe to conclude therefore that the factor that renders intact rats liable to this anæmia is a dietary one The present series of experiment is not sufficiently conclusive to decide the exact nature of the deficiency, the results suggest that either a relative deficiency of vitamins A and C or some other closely associated dietetic factor is most likely to be responsible

Previous writers have not reported the occurrence of Bartonella organisms in intact albino rats though Bartonella bodies have been recorded in the red cells of wild rats and other rodents unassociated with an anæmia For reasons quoted in the literature the anæmia produced by splenectomy in most strains of rats is considered an infective one due to the presence of this organism Up to date it has been impossible, with some possible exceptions (Bayon, 1928 and Mayer, 1921), to culture this species though the allied *Bartonella bacilliformis* has been grown But if it is assumed that it is an infective anæmia then the removal of the spleen, i e, a large bulk of the reticulo-endothelial system, enables an infection which is latent to become manifest By dietetic means alone it has been possible to produce the same effect with resultant anæmia in typical form In this connection the work of Rosenthal (1924) and his co-workers and Regendanz and Kikuth (1928) on the importance of the reticulo-endothelial system in the defences of the body, especially in protozoal infections, is of considerable importance They consider that the trypanocidal power of normal serum is not a direct function of the serum but that some element in it is activated in the body of the experimental animal by some element in the latter's reticulo-endothelial system If the reticulo-endothelial system is knocked out either by removal of a large portion of it, i e, the spleen, or by blocking with saccharated iron this power is lost (normal serum also is inactive *in vitro*) If then a large part of the rat's defence mechanism against a Bartonella infection, whatever the nature of the Bartonella organism may be, lies in the spleen, an assumption warranted by the results of splenectomy, what is the action of the dietetic deficiency? Is it not possible for the deficiency under consideration to have a specific action on the reticulo-endothelial system even as a vitamin A-deficiency has a specific action on the epithelial structures,

rendering the animal peculiarly liable to bacterial infections (Tyson and Smith, 1929 and Wolbach and Howe, 1925)? Post-mortem, there are abundant signs of changes in the reticulo-endothelial system but whether they resulted from the diet and occurred before the infection developed or are a result of the infection it is impossible from the present work to say All that can be stated is that by dietetic means alone a Bartonella anæmia has been produced in the intact rat and that this is possibly the result of changes in the reticulo-endothelial system due to the faulty feeding

It should be noted that the female animal is particularly liable to this anæmia and that the disease is peculiarly fatal in the pregnant animal

Liver, spleen and vitamin A therapy in splenectomized rats failed to afford any protection against this anæmia It is possible that in the intact animal they would have had some protective or curative effect a large scale experiment would be necessary to test this

Summary

(1) A severe anæmia has been produced in rats by feeding on diets devised to be relatively deficient in vitamins A and C

(2) The deficient diets included both natural and synthetic diets

(3) The anæmia was more frequent and much more severe in female animals

(4) Many of the animals made spontaneous recoveries

(5) Examination of slides demonstrated the presence of *Bartonella muris* in the red cells of the anæmic rats the anæmia was therefore 'pernicious' or 'Bartonella' anæmia occurring spontaneously in intact animals

(6) Splenectomy showed that all the Coonoor stock was infected with Bartonella

(7) Liver and spleen feedings failed to protect the rats from this anæmia after splenectomy

(8) The only animals in our experiments that developed an anæmia after the inoculation of Bartonella infected blood were pregnant rats fed on the so-called Bombay Hindu diet

In conclusion we would like to thank Colonel R McCarrison and the staff of the Nutritional Research Laboratories for their very generous help in this work

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APPENDIX

Splenectomy in rats

The skin over the left side of the abdomen and the left flank is shaved, cleaned and painted with iodine The animal is anæsthetized with open ether given from a cone (card-board) lined with wool When well under it is placed on its back on a board and its legs extended by means of tapes attached to pegs An incision about 1¼ inches long is made from just below the angle of the ribs downward in or just in front of the left flank The proposed site of the incision in the abdominal wall is first caught up by sutures one at the upper and the other at the lower end The skin and abdominal muscles are severed and when the peritoneum is reached gentle traction is made upon it by the two sutures so as to raise it well above the underlying viscera A curved bistoury is inserted at the lower end of the wound and a small opening made to admit blunt-pointed scissors with which the opening into the abdominal cavity is completed The spleen frequently presents in the wound, if it does not the anæsthetist presses gently from behind when it practically always appears in the wound The spleen is lifted up by a pair of forceps and the pancreas separated from it, the splenic vessels running between the two organs are then divided by means of the blunt-end of a scalpel heated to a dull red heat The greatest care must be taken to heat to the right temperature as too great a heat cuts the vessels cleanly and causes severe hæmorrhage and insufficient heating causes tearing and hæmorrhage again Should bleeding occur it can be controlled by touching the point with wool dipped in absolute

alcohol touching the pancreas with the cautery leads to further hæmorrhage The peritoneum is sewn by with a continuous, fine catgut suture and the skin by a continuous cotton suture The wound is swabbed with iodine and the animal returned to a cage lined with wool to prevent injury during recovery from the anæsthetic

The strictest antiseptic precautions should be taken In our series there was no trouble from sepsis, the wounds healed rapidly, the rats removing their own stitches but never before the wound had healed, and post-mortem there was very little scarring of the tissues The operative death-rate in our series was 1 in 67 animals

It should be noted that during the operation the spleen is engorged with blood Chart 3 shows the weights of the spleens removed by operation the regression line on the same chart is from values obtained from normal animals belonging to the same stock, but removed post-mortem after death from a blow on the head The increased weight of the organ removed at operation is due to the engorgement with blood that occurs during anæthesia It has been shown before that the weight of the spleen depends on the mode of death, hence for comparative studies it is essential that the animals should be killed in the same manner and spleens removed under an anæsthetic cannot be compared with those removed post-mortem

Explanation of Plate XLIV

Fig 1 Blood film from normal rat × 250

„ 2 Blood film from intact rat with severe Bartonella anæmia × 250

„ 3 Blood film from splenectomized rat with severe Bartonella anæmia × 250

„ 4 Blood film from splenectomized rat Red cells show Bartonella 'organisms' × 1,000

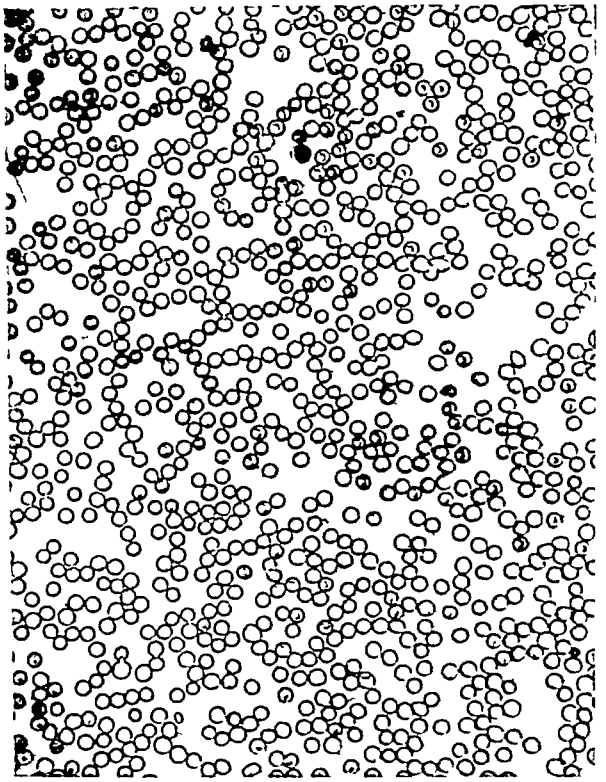

Fig 1

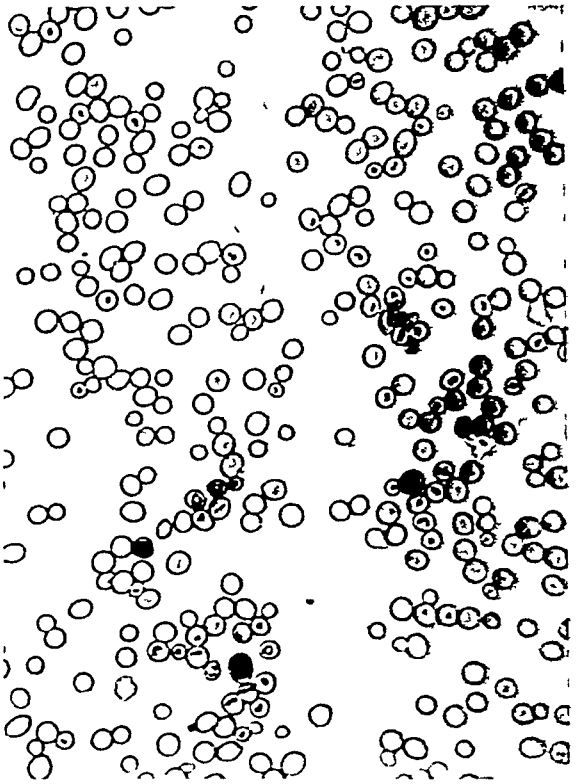

Fig 2

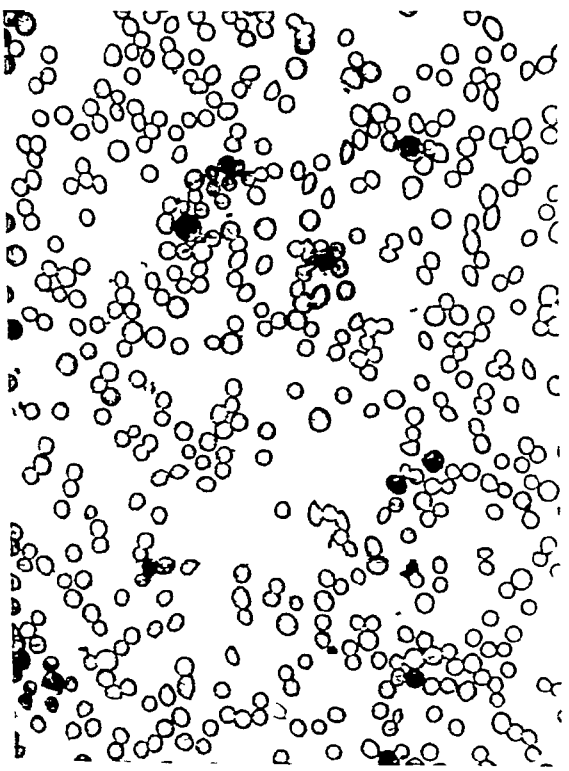

Fig 3

Fig 4

SEASONAL VARIATION IN HOOKWORM INFECTION

BY

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As the result of an investigation in a Calcutta gaol, Chandler (1926) made the statement that hookworm infection was rapidly lost, when reinfection did not occur He assumed that all prisoners on admission to the gaol had a fairly constant average infection rate He divided the prisoners into batches depending on the length of time the men had been in gaol, and he worked out the average egg count of each batch He obtained a rapidly falling curve, which showed a close correlation with the length of time the prisoners had been in gaol Up to this time it had been the general opinion that hookworm infection was slowly acquired and correspondingly slowly lost, and a paper by Smillie (1922) was considered to have proved this It was decided to check Chandler's observation as, if correct, it was of considerable importance

The same gaol was chosen for the investigation, but the subject was approached in a different way All new admissions to the gaol were examined, and after about a month fifty-six prisoners with fairly heavy hookworm infections were found, and these were selected for further observation, their stools being examined once a month for a period of twelve months, from November 1927 to October 1928 Unfortunately, owing to transfer to other gaols, the number of the prisoners available gradually diminished so that towards the end only half of them were left and the figures are rather small, they are given in Table I

It will be seen from these figures, from which Chart 1 has been compiled, that there is a rapid loss of infection during one period of the year, but that this period is preceded by one of equally rapid gain This suggested that, for part of the year at least, the prisoners were acquiring infection Careful inspection of the gaol and of its sanitary routine did not reveal any possibility of the prisoners becoming infected within its precincts This is the same

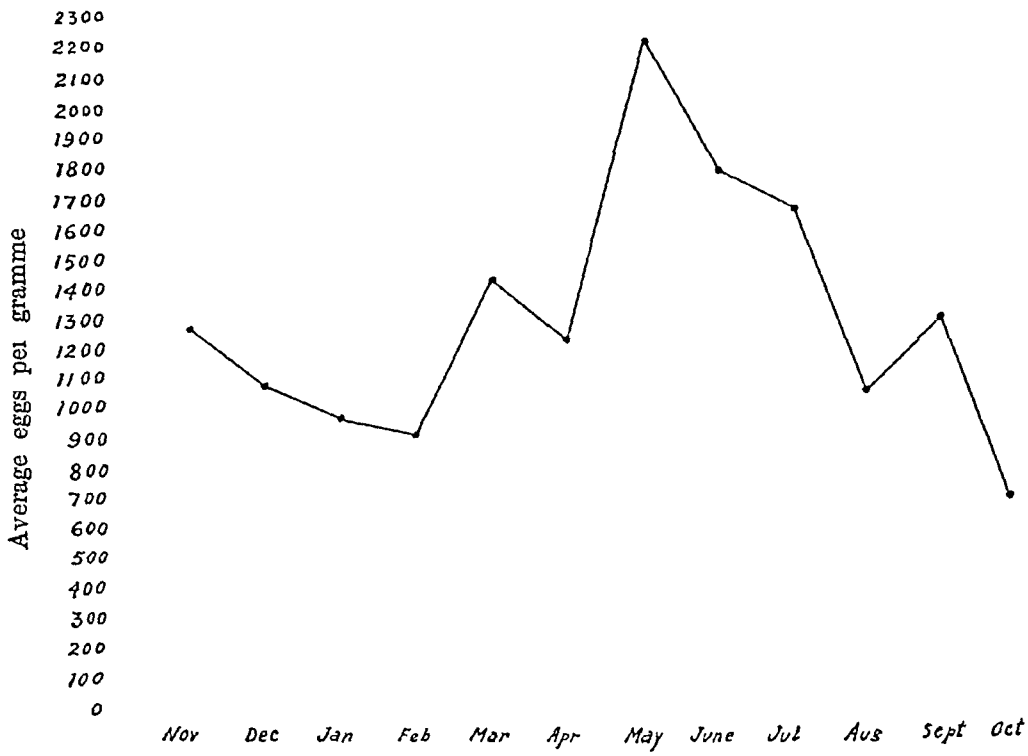
TABLE I

Egg counts on the same prisoners for twelve months

Month	Number of prisoners	Average eggs per gramme per prisoner
November	56	1,271
December	56	1,089
January	56	980
February	53	928
March	50	1,132
April	16	1,243
May	41	2,222
June	39	1,803
July	34	1,671
August	31	1,071
September	28	1,328
October	26	723

CHART 1

Hookworm egg counts in a Calcutta gaol



conclusion at which Chandler arrived, but further inquiry revealed the fact many of the prisoners are sent out of the gaol daily to engage in horticultural and other work This appears to be the only possible way in which infection could have been acquired, and it also serves to explain how some of the prisoners examined by Chandler had infections after being in the gaol for many years At the same time the gaol authorities do not consider infection likely to occur,

while the prisoners are engaged in outside work, as no night-soil is used in the gardens where they are employed

As the numbers available were so small it was possible that the variation observed was only accidental, and so Lieut-Col A D Stewart, I M S, kindly examined the figures statistically, and although the true variation is less than that shown by the simple averages in the table, he found that the variation is significant

When Chart 1 is examined it is seen that the increase occurs in the pre-monsoon period, and that once the monsoon is properly established the infection rate rapidly falls, and that the fall continues throughout the wettest months of the year But as the number of people examined was so small and as they were living under abnormal conditions compared with the free population, it was thought that these findings might be due to some unrecognized factor, which was not present in people living elsewhere, and not under restraint It was accordingly decided to continue the inquiry in other localities and among free people

Two tea gardens in the Dooars of Northern Bengal were made available by their managers, for repeated observation over a prolonged period, and stools from the same individuals in these gardens were examined on five occasions, viz, in June 1928, in November 1928, in March 1929, in June 1929, and in November 1929 During the period of observation no hookworm treatment was carried out among these coolies All the stool collections were made under the supervision of one of my assistants, who himself measured them all and placed them in bottles of Antiformin after the method of Maplestone (1929), and the egg counts were done by the writer and his senior assistant In this way variation in results owing to the differences in method and capability of the people employed were eliminated It will be noted in Table II that there is

TABLE II

Egg counts on the same persons on five occasions, on two tea gardens in the Dooars

	June 1928	November 1928	March 1929	June 1929	November 1929
Number examined	784	685	635	582	530
Number infected	726	586	511	554	471
Per cent infected	92·6	85·5	80·4	95·2	88·8
Average eggs per gramme of total examined	1,258	653	526	1,002	577

a gradually diminishing number of people examined on each occasion, but the averages obtained were carefully checked, and there was no evidence that the

successive decrease in numbers had any bearing on the results People of all ages and of both sexes were included, and it was noted that none of the variation noted in egg counts could be explained by any alteration in the proportion of persons of different ages or sex, as the numbers lessened Approximately half the people were from each of the two gardens, and as each garden showed

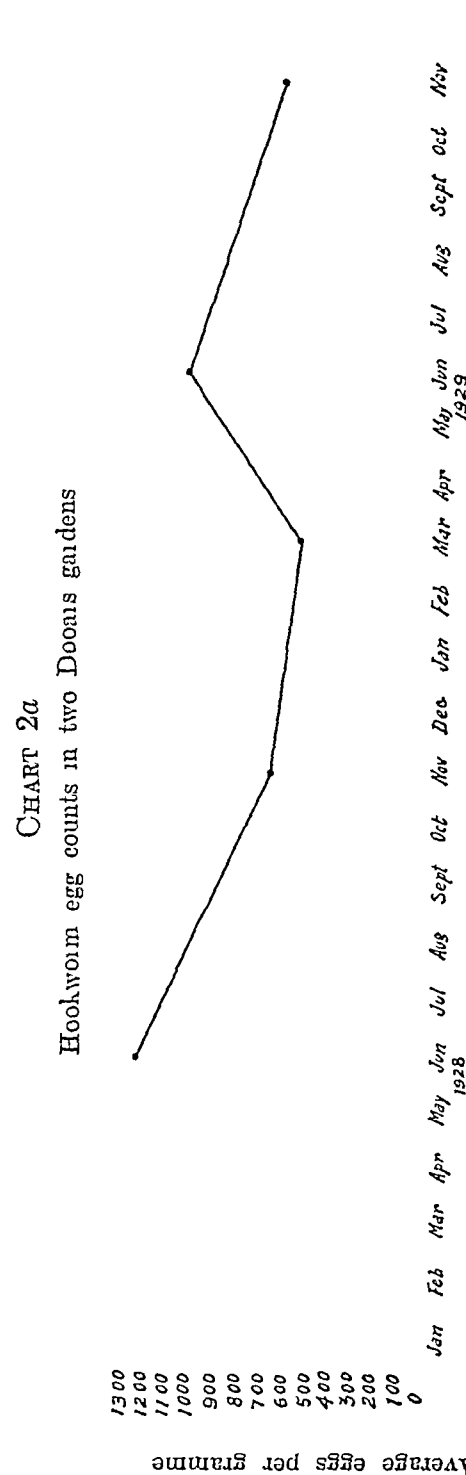


CHART 2a
Hookworm egg counts in two Dooars gardens

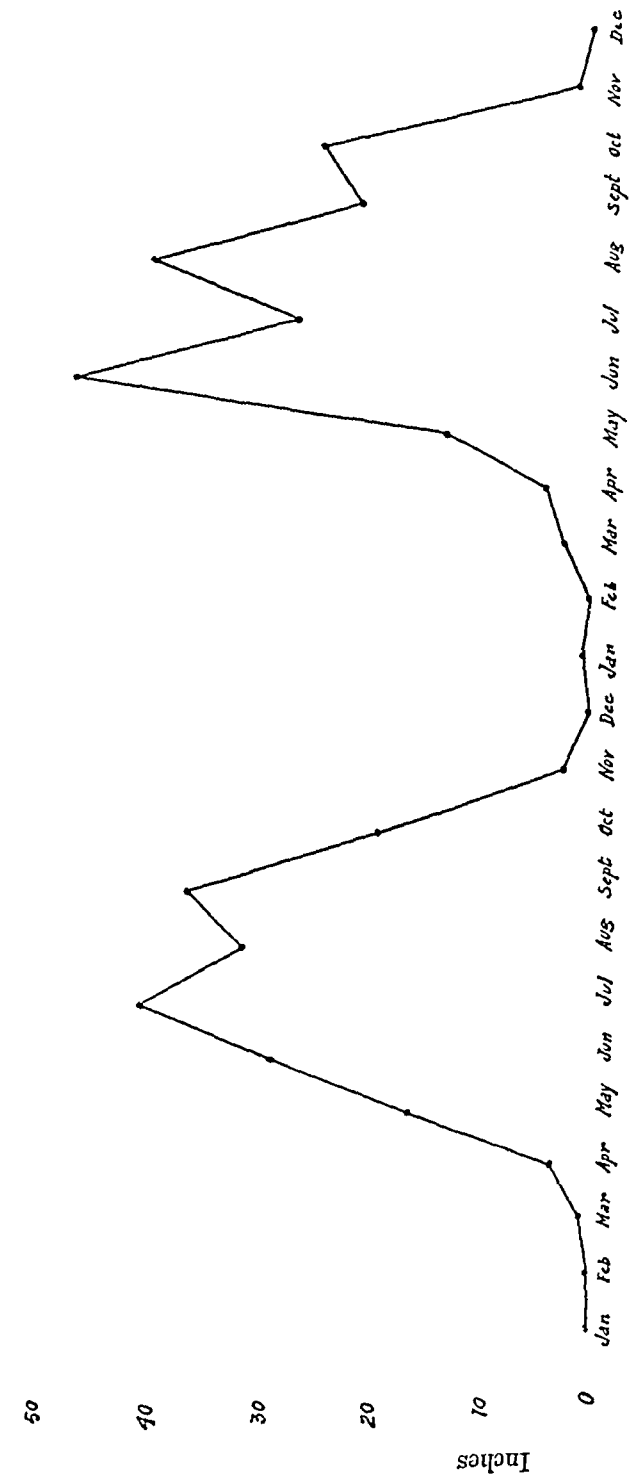


CHART 2b
Rainfall in the Dooars gardens during the observation

about the same infection rate on each occasion, the results have been given as a total of the two gardens, though they were kept separate during the course of the work

These figures show that there is a definite fall in the egg output in the monsoon period of each year, and if Chart 2 is consulted it is brought out that the falls on each of these occasions were nearly parallel It will be seen that the egg count for June 1929 is somewhat lower than in June 1928, this may be only a matter of chance, but study of the rainfall for the two years shows that in 1929 there was more rain in March and April and that it was much heavier in May 1929, than in the corresponding months of 1928 But what is probably of more importance than the amount of rain is the number of days on which rain fell, for this would mean more days on which there was too much moisture for freshly hatched larvæ to live * It is found that the number of days on which rain fell in March, April, and May 1929 was 6, 18 and 24 respectively, against 4, 9, and 18 for the same months in 1928 Therefore the lower count in June 1929 compared with June 1928 is in accord with the general observation of this paper, that too much rain is unfavourable for hookworm development Of course it is not known at exactly what time of the year the highest infection rate occurred, so it is not clear if the maximum had been reached in June, or whether it was a little before or after this month It is interesting and confirmatory that the alteration in average egg count represents actual alteration in the number of worms harboured, when it is noted that the percentage of persons infected follows the amount of infection demonstrated by the average egg count

In reading all the Tables and Charts in this paper it is important to remember, that what is shown in an egg count represents what has occurred to the individual, six weeks to two months previously, in respect of acquiring infection On account of the long intervals between examinations in the present series it is not possible to apply this principle, but as far as they can be compared these results appear to be in agreement with those in the Calcutta gaol

The next series of examinations were undertaken in two tea gardens in the South Sylhet district of Assam The method employed in this instance was to collect one hundred stools from each of the gardens, as nearly as possible once

* Maplestone (1926) in experiment 3*a* shows that only a little over one-third the number of larvæ are produced from freely moistened soils compared with controls, which were kept just damp In the course of these experiments, the detail of which is not given in the original paper, ten cultures kept heavily moistened together with an equal number of controls were put up One of the cultures and one control were examined daily for the number of larvæ present, beginning twenty-four hours after they were put up, and it was found the eggs hatched just as well in the very wet cultures as they did in the controls, for the number of young larvæ extracted from each were approximately equal on the first day But on each successive day, until the larvæ became sheathed, the number extracted from the wet cultures steadily diminished After the sheath was formed the larvæ lived as well in very wet earth as they did in slightly moist earth This experiment was performed on several occasions, and the results were always the same

a month, the result was that ten examinations were performed between February 1929 and February 1930 Stools from the same people could not be obtained on each occasion, but as the population of one garden was about 400 and of the other about 1,500, it is certain that many of the people were examined more than once The stools were placed in Antiformin and were sent to Calcutta where they were counted by the same people throughout the Inquiry

Chart 3 has been compiled from the figures in Table III, and Chart 4 is the monthly rainfall in these gardens during the period of the investigation

TABLE III

Egg counts on 100 *persons from each of two tea gardens in South Sylhet* (1929-1930)

Month	Number examined	Garden 1 (Pop 400) Av epg	Garden 2 (Pop 1,500) Av epg
February	100	875	300
April	100	624	365
May	100	1,074	730
July	100	1,484	722
August	100	1,394	627
September	100	896	423
October	100	1,012	534
November	100	670	495
January	100	652	255
February	100	376	158

Comparison of these charts shows that the rise in infection begins in the April-May period and that it continues until July, and that the fall begins in the July-August period, that is, about two months after the rains commence the rise begins, and that the fall begins about two months after the period of maximum rainfall is reached If these comparisons are still more closely analysed, it is found that the second half of July was exceptionally dry for the monsoon season, there being only about three inches of rain in this fortnight, and this is reflected in a slight rise in the infection rate in both gardens in the September-October period This observation is possibly only due to chance, but at the same time it is quite in accord with the other findings, that moderate rain means increase and heavy rain means decrease in infection

From these observations it appears, that in the localities so far examined the principal factor in limiting the amount of hookworm infection is the

excessive rain in the monsoon and not the dry time of the year, and that the only time of the year in which the amount of infection increases is a short one of about two-and-a-half months in the pre-monsoon season, when there are infrequent showers which moisten the ground occasionally and render it

CHART 3

Hookworm egg counts in Sylhet gardens

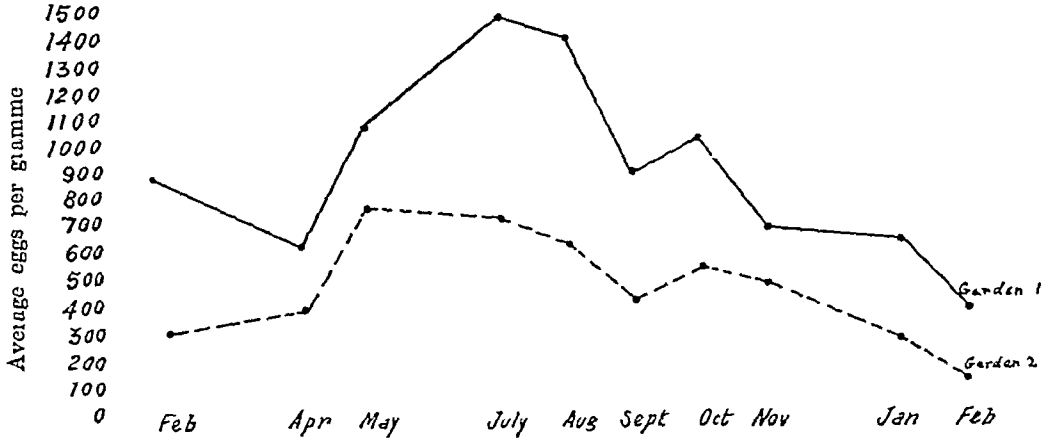
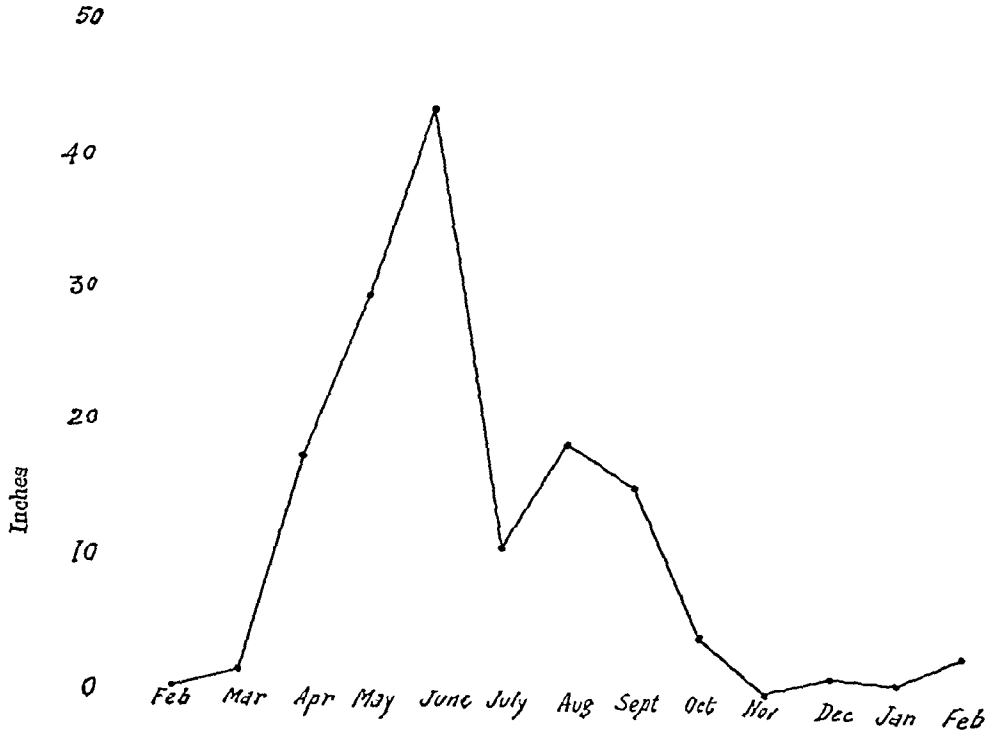


CHART 4

Rainfall in Sylhet during the time of observation



favourable for the maximum development of hookworm larvæ Continued examination of a community shows that at no time of the year does hookworm infection cease altogether, but that some individuals are losing their infections either wholly or in part, and that others are acquiring infections or adding to their existing infections, and that this change is continually going on Therefore the real meaning of the statement that hookworm infection is being acquired during one part of the year and is being lost during another part is, that when the losses and gains of a number of people are added together it is found that the total is greater at one time than at another In other words, the balance in favour of hookworm in the present investigation exists for about two-and-a-half months of the year, and it is against the hookworm for the remainder

Although the maximum temperatures are always high enough for the favourable development of hookworm larvæ in Bengal and Assam, the minimum temperature falls below 60°F for a considerable period every year This temperature is recognized as the critical one for development of hookworm larvæ, for below 60°F the development is much retarded, and a further fall of a few degrees would in all probability prevent it altogether In Calcutta during the cold season 1927-1928 the minimum temperature was above 60°F only on one occasion between the 9th December and the 18th January, while before and after this period the temperature only fell below 60°F on a few isolated occasions Reference to Chart 1 shows that the definite increase in egg output began some time between February and March, that is approximately six weeks after the minimum temperature became fairly regularly established above 60°F

In the tea districts, the period during which the minimum temperature remains continuously below 60°F is much longer For example in Sylhet, from the 12th November, 1928 to the 13th March, 1929 the minimum temperature was above 60°F only on eleven occasions, and from the 10th December, 1928 to the 23rd February, 1929 it was below 50°F for fifty-eight out of the seventy-six days Reference to Chart 3 shows that the increase in egg output began during the April-May period, that is six weeks or more after the minimum temperature remained permanently above 60°F

In experiments, such as those of McCoy (1930) who worked with *A caninum* larvæ, in which the effect of lowered temperatures on the larval development were investigated, the temperatures were apparently maintained permanently at any given level, but in actual practice in Bengal and Assam there is a daily range of about 20 degrees during the cold season It seems probable that the daily alteration from a favourable to an unfavourable temperature for development of larvæ would have a much more harmful effect than a continuous slightly unfavourable one

The rainfall in Calcutta was nil from November 1927 to March 1928, but the rise in infection rate began in February-March The reason for this is that the prisoners were working on irrigated ground so they became infected irrespective of rainfall Also in Garden 1 in Sylhet, owing to the special condition

of the coolie lines and defæcation areas, which will be explained in detail below, the soil was in a highly favourable state for larval development throughout the dry and cold months of the year, but there is no sign of an increase of infection until six weeks or more after the minimum temperature became stabilized above 60°F The temperature in the Dooars is much the same as in Sylhet, but owing to the length of time between examinations in this district it is not possible to ascertain exactly when infection began to increase

Owing to the unusual conditions of the soil during the dry season in the two localities where it has been possible to examine the effect of the low temperatures, it has not been possible to estimate the effect of the absence of rain in lessening the amount of infection This is probably of importance in the case of the gaol for the rise in hookworm infection begins a month before there is any rain at all But in Sylhet, where the period of low minimum temperature is much longer, the onset of occasional rains and the rise of the minimum temperature to a permanently favourable height roughly coincide, so it is not possible to separate the effect of the low temperature and the dry weather in this case

The rise in infection rate during the pre-monsoon period, when the rain is intermittent, indicates that slight moisture is the most favourable condition for larval development, so one would expect a similar rise at the end of the monsoon, when the rain again becomes infrequent and the soil slightly drier There is no evidence in the figures so far obtained that this condition occurs, and the explanation offered is, that as the rains decrease the minimum temperature begins to fall, and the two factors coming together tend to balance one another so that the amount of infection remains more or less on a level during this period It is probable, that in other places where there is no cold season, there would be just as definite a rise in infection rate at the end of the wet season as there is at the beginning

If reference is made to Table III it will be seen that Garden 1, with a population of about 400 in its lines, has an infection rate approximately double that of Garden 2 throughout the year, and the latter garden has a population of about 1,500 This is contrary to what one would expect, for both gardens are under the same medical and general administration, they contain coolies of the same tribes, and they are within two miles of each other Examination of the coolie lines in these two gardens, however, revealed a difference in their situation and arrangement In Garden No 1 the huts are all crowded together on a short narrow ridge, and on each side of the ridge there are small low flats, where defæcation is performed and the concentration of fæces is considerable These flats are permanently moist from seepage from the surrounding higher ground, and they are not scoured during heavy rains In Garden 2 the huts are scattered over a wide area occupying several steep ridges, with only a few huts on each ridge, and defæcation is performed in the narrow steep gullies between These gullies are dry for the most part, and are subject to scouring in the rains, owing to their steepness This is a

valuable example, as it clearly demonstrates how a little care and knowledge applied to the selection of coolie lines might greatly lessen hookworm infection, without any other measures being employed

Other helminths

The eggs of the other common intestinal worms present were counted at the same time, except in the gaol where the infections were so few that it was not worth while

TABLE IV

Average eggs per gramme of Ascaris and Trichuris in the Dooars

	JUNE 1928		NOV 1928		MARCH 1929		JUNE 1929		NOV 1929	
	Asc	Trich	Asc	Trich	Asc	Trich	Asc	Trich	Asc	Trich
Number examined	784		685		635		582		530	
Number infected	736	601	618	559	511	530	169	469	395	427
Per cent infected	94	77	90	82	80	83	81	81	75	81
Average e p g per total	7,396	302	8,220	290	7,401	304	5,229	270	6,864	186

A glance at Charts 5 and 6, compiled from the above tables, shows that there is no evidence of seasonal variation in the infection rate of these two parasites This is what one would expect, for the eggs of these worms will remain unhatched and alive in water for prolonged periods, so they are not likely to be killed by heavy rainfall An important point brought out by the absence of loss of infection by these two worms during the wet season, is that the amount of infection is not apparently effected by dilution of fæces and scattering of eggs by rains This supports the view, that in the case of hookworm, the loss during the wet weather is due to actual death of larvæ, and not to them being more widely, and consequently more thinly, distributed by the heavy rain

In the Dooars, infection with a species of *Trichostrongylus* is fairly common Infections with this worm seem to be invariably light, and it is probably of little practical importance, but it is interesting to note that it shows a variation similar to that of hookworm The counts made in June and November 1929 are slightly higher than those in the same months of 1928, but the number of infected persons is small and the counts are as a rule below 100 eggs per gramme, so the total is markedly affected by one or two abnormally high counts, which actually did occur in 1929

TABLE V

Average eggs per gramme of Ascaris and Trichuris in Sylhet

	Number examined	Garden 1		Garden 2	
		Asc	Trich	Asc	Trich
February	100	12,987	156	3,313	142
April	100	1,387	74	2,992	102
May	100	2,142	133	6,051	252
July	100	1 844	71	3,487	148
August	100	1,786	101	2,968	134
September	100	1,077	56	2,356	121
October	100	1 122	65	6,175	216
November	100	3,282	100	2,798	215
January	100	7 787	95	4,261	158
February	100	9,103	216	4,100	108

CHART 5

Ascaris and Trichuris egg counts in Dooars gardens

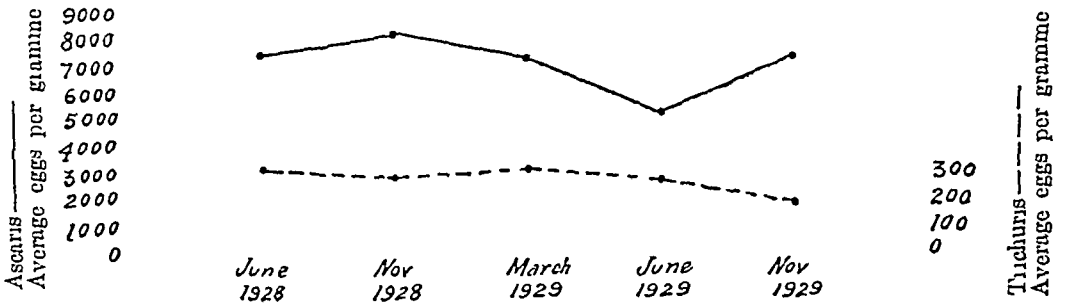


TABLE VI

Average eggs per gramme of Trichostrongylus in the Dooars

	June 1928	Nov 1929	March 1929	June 1929	Nov 1928
Number examined	784	685	635	582	100
Number infected	143	106	57	108	18
Per cent infected	18·2	15 4	9·1	18·5	25
Average e p g per total	24	23	14	28	530

The alteration in egg output of this worm is slight compared with that of hookworm, but the change follows the same seasonal variation This worm belongs to the same group as do Ancylostoma and Necator so it is probable that it has a similar life history, and it is interesting, that what change occurs in the infection rate agrees with the change in the hookworms

CHART 6a

Ascaris egg counts in Sylhet gardens

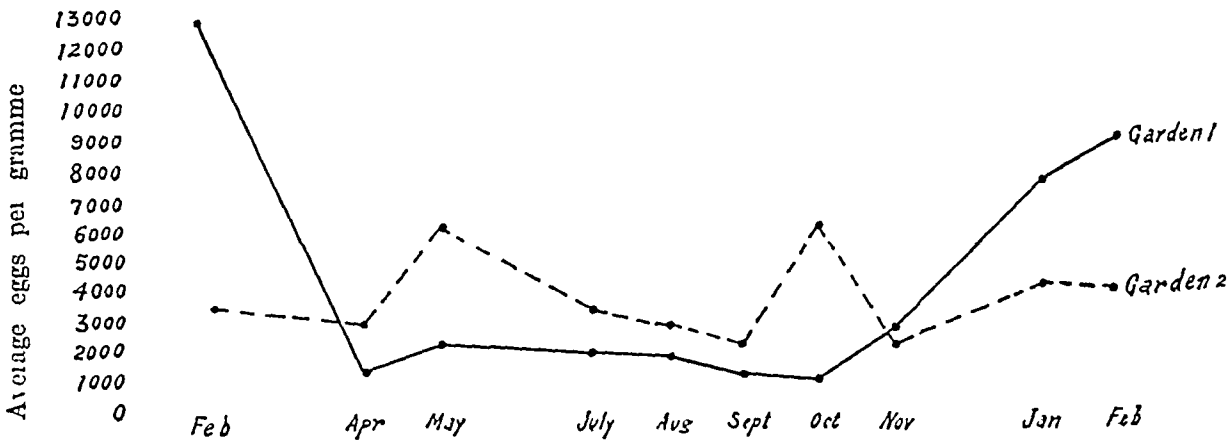
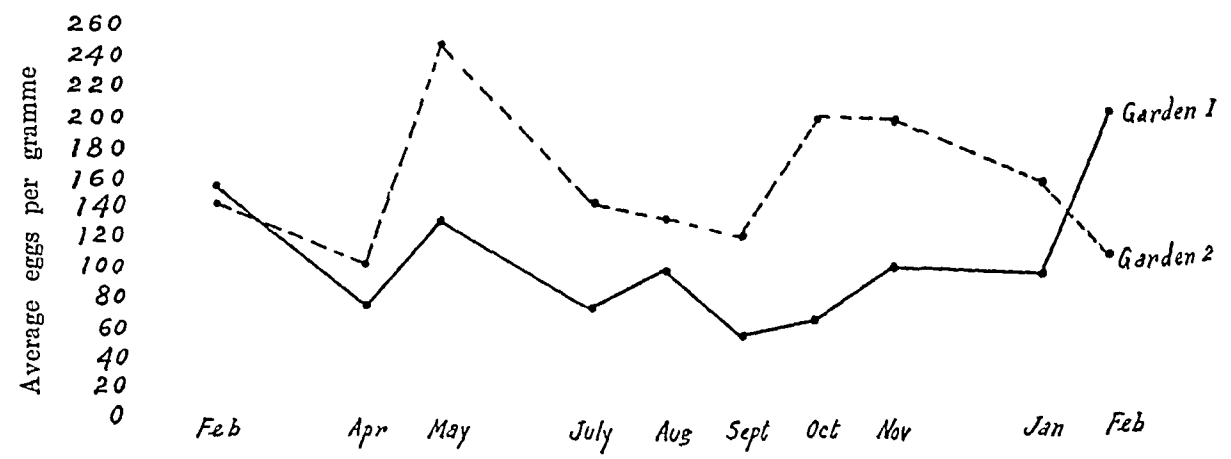


CHART 6b

Trichuris egg counts in Sylhet gardens



DISCUSSION

It has been shown experimentally, by Payne (1922) and by Maplestone (1926), that excess of moisture is deleterious to hookworm larvæ, and various writers, notably Cort, etc (1926), and Sweet (1927), have expressed the opinion that hookworm infection is not so heavy where the soil is very wet, but as far as the writer is aware this is the first occasion on which the actual amount of loss in infection due to the wet season has been shown in any country Cort, etc (1929) examined one hundred and seventy-five persons in a Panama village

on three occasions, viz, at the beginning, in the middle, and at the end of the wet season The conclusion these workers came to was that there was no significant change during this time, and as a corollary, that there was no loss of infection during the extremely dry season to which this locality is subjected They accordingly do not agree with Chandler's conclusion that hookworm infection is rapidly lost in an average dry season If then Table I is examined, however, in the light of the present paper, it will be seen that what slight change does occur, agrees with my own figures, for there is a slight increase between the first and second counts, that is during the early part of the wet season, and there is a slight drop between the second and third counts, that is during the latter half of the wet weather But the final result is that the difference between the first and third counts is negligible, therefore on the data available there appears to be no definite seasonal variation in infection rate in Panama, but it is possible that more frequent observation of larger numbers of people might show some change to occur

In the case of Ascaris, the Panama workers find a definite increase during the wet weather, and from this they deduce that there must be a corresponding loss in the dry weather From some observations the writer has made he is inclined to agree, that there is probably a rapid loss of Ascaris infection, but in the observations recorded in this paper there is no evidence that the change from wet to dry seasons in Bengal and Assam have any influence on it

Although a considerable change in infection rate of any helminth from one season to another is suggestive that there is a rapid loss and gain in infection at different times of the year, this state of things need not necessarily be occurring for in an endemic country where the inhabitants are continually losing and acquiring infection the worms they lose this year may have been picked up many years ago and the ones they gain this year may remain in their intestines for many more years, so the seasonal variation that goes on may be an annual reflection of what occurred an unknown number of years before

Augustine and Smillie (1926) showed that in Alabama hookworm infection is not so severe among the inhabitants of the heavy black soil areas, as it is among the people living on sandy soils They ascribe this difference to the fact that in the dry season the black soil becomes baked very hard so hookworm larvæ cannot retreat into the deeper layers, nor can they move about in it It is possible that the factor of greater retention of water by the black soil during the wet season also plays an important part in destroying the larvæ

Conclusions

1 An isolated examination of a sample of any population for the degree of hookworm infection is unreliable unless the seasonal variation is known It is also unsafe to assume on theoretical grounds what is likely to be the time of heaviest infection For instance Chandler (1926-28) in his numerous

papers in hookworm infection in India has applied corrections to his figures on the assumption that November is the time of maximum infection, and April and May about the minimum It is shown in the present paper for Bengal and Assam at all events, almost the reverse appears to be the case

2 The present investigation only applies to certain areas in Assam and Bengal, and the different results obtained by Cort and his co-workers in Panama, both with regard to hookworm and ascaris, indicate that much more work of this nature must be done before a working formula can be produced, which could be applied to any place to estimate the seasonal variation in these infections, being given the conditions of rainfall, soil, climate, etc

3 In the places investigated, intensive sanitary work in the nature of night-soil conservancy for the short period of about two-and-a-half months (March, April, May) each year would probably reduce hookworm infection in these areas to a negligible degree

4 A striking object lesson has been given as to how selection of coolie lines might favourably influence the amount of hookworm infection

In conclusion I wish to express my thanks to W J Gray, Esq, of Ramchenra, J Haigh, Esq, of Sylee, Dr E M Rice and his successor Dr Lawrence Hunt of the Lungla Valley Tea Company for the facilities and assistance they afforded to me in carrying out this work

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THE ARTIFICIAL FEEDING OF SANDFLIES

BY

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INTRODUCTION

IN carrying out kala-azar transmission experiments the sandflies have been infected by feeding them on kala-azar patients, or infected hamsters, usually the former. All the present writer's attempts to make sandflies of the species with which he has been working, namely *P argentipes*, feed through a membrane have entirely failed. No useful purpose would be served by repeating in detail the methods by which this was attempted, save to state that young rabbit's skin, mouse's skin and rabbit's gut were used as membranes, a variety of fluids, all of them containing a generous admixture of blood corpuscles or hæmoglobin, were used, and a scheme by which the blood was kept constantly moving and at 37°C. while the flies were in a cooler atmosphere was devised. With the methods described by Adler and by Hu and Lee (1928) we had no success whatsoever.

For studying the behaviour of *Leishmania donovani* in the sandflies *P argentipes* or *P papatasii*, the sandflies could be fed upon infected patients, the patients are plentiful here and the feeding flies cause them little discomfort. But for studying the behaviour of other species of leishmania or other flagellates in these sandflies, or for studying the behaviour of any flagellate in the sandfly, *P minutus*, which in our experience will not feed on man under artificial conditions, it was necessary to introduce some other means of feeding the flies. The technique devised and described by Hertig and Hertig (1927) seemed to offer the best opportunity.

Their technique does not appear to have been described anywhere in any detail, and from the description given in the above-quoted paper the present writer would have had considerable difficulty in making the apparatus had he not also had an opportunity of seeing a specimen of it at the 7th Congress

of the Far Eastern Association of Tropical Medicine in Calcutta (1927) It was found that certain modification both in the apparatus and in the technique were advisable Furthermore, certain details in the technique which were entirely omitted from the originator's description had to be supplied The present writer, therefore, feels that a more minute description, both of the apparatus and of the technique, might at some future time be of value to workers in this or similar fields

The apparatus—By means of this apparatus sandflies can be made to ingest any fluid substance The two essential parts of the apparatus are the vice, by means of which the fly is held, and the fine capillary pipette with a narrow aperture which is passed over the proboscis of the insect

The vice—The vice in its simplest form consists of a cylinder of cork, 1 6 cm in diameter and 3 cm long, which has been split longitudinally (Fig 1, A, *v*) A strip of metal foil (Fig 1, A, *s*) with two sharp ends is bent into the shape of a 'V' and the two points thrust into the end of the cork cylinder, one end into each half of the split cylinder, in such a way that the metal foil acts as a spring keeping the two halves of the cylinder apart until they are approximated by digital pressure, the apparatus thus constitutes a pair of forceps with a flat cork end by which the wings of the fly can be gripped The whole circumference of the distal one-third of the cork cylinder is then pared down so that when closed it fits tightly into a glass cylinder (Fig 1, *t*) of 1 1 cm diameter (internal) In actual practice it has been found more satisfactory to make this vice out of cork and wood, as shown in the illustration, instead of cork alone The wood gives the necessary rigidity and strength, whereas the cork has sufficient elasticity to prevent the wings of the fly being injured, this does not in any way complicate the preparation of the vice, a small piece of cork sheeting is glued to each inner surface of the longitudinally split wooden cylinder and the whole pared down to the necessary shape

This glass cylinder should be 3 cm long by 1 1 cm internal diameter with both ends ground, there is no great disadvantage if one end is welded, as slight narrowing of the diameter at one end would not be a serious matter, but it is essential that this should not occur at both ends, and on the whole it is better to have both ends ground When the cork cylinder is thrust into this tube the two halves of the cylinder are held closely together, so that the fly is gripped after digital pressure has been withdrawn (Fig 1, B) A cork ball (Fig 1, *cb*) is then prepared which also fits exactly into this tube Along the axis of this ball a hole is made and the feeding pipette (Fig 1, *fp*) is thrust through this The ball with the pipette are now placed in the opposite opening of the glass tube The pipette should be held firmly in the ball, but the latter should fit into the glass tube in such a way that the pipette, whilst freely moveable in all directions, will remain in the position in which it is placed

The feeding pipette—The pipette should be about 6 cm long and less than 1 millimetre external diameter, one end is fused in a small Bunsen flame until

the opening is exactly the right size The preparation of the pipettes is one of the most tedious parts of the whole procedure There appears to be no short cut and the only method is to do it by trial and error First of all it is essential that the end of the tube be cut off absolutely square by the aid of

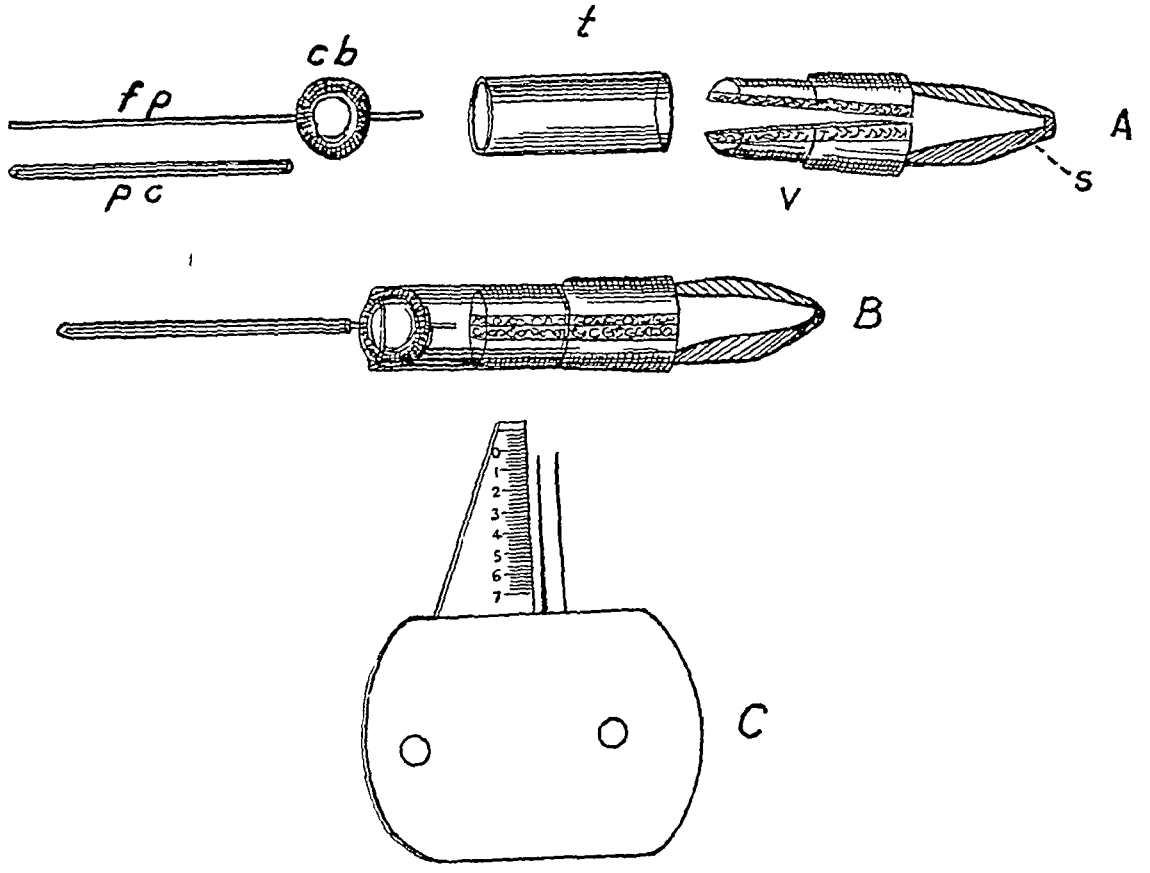

Fig 1

A The separate parts of the apparatus
s folded metal foil forming a spring
v cork and wood vice
t glass tube
cb cork ball
fp feeding pipette
pc pipette cover
B The parts of the apparatus assembled
C Gauge for measuring pipettes

a very sharp glass cutter It is best to carry out this operation with the aid of a pair of magnifying binoculars or a watch-makers magnifying glass

Gauge for standardizing pipettes—For the purpose of standardizing the pipettes the present writer had a small gauge (Fig 1, C) prepared, this

consists of a tapering wire welded to a flat piece of brass with a scale also welded to the same piece of metal, parallel with it The markings on the scale are purely arbitrary, but the gauge of the wire at each marking on the scale can be measured by means of a micrometer and a table prepared To measure the calibre of the opening, the wire is passed gently into the pipette as far as it will go and a reading made at the point to which it reaches If the calibre is too large further heating in the Bunsen flame is necessary, but if it is too small the end of the pipette will have to be cut off and another attempt made About 0 1 to 0 12 millimetre has been found the most satisfactory calibre for the feeding pipettes for *P argentipes* and *P papatasii*, and for *P minutus* a slightly narrower calibre, but we have not found the exact size of the opening of the pipette a matter of primary importance

Finally, another fine tube, 2 cm long with a calibre of 1 mm (Fig 1, *pc*) is prepared, and one end sealed, this is placed over the open end of the feeding pipette to ensure absolute asepsis whilst the pipette is being passed over the proboscis of the insect

The success of the operation depends very largely on the accuracy of the fitting of the cork ball, if this is too loose the pipette may move after it has been placed in position, and if it is too tight great difficulty will be experienced in getting the pipette over the proboscis of the fly and the pipettes will frequently get broken

The operation—The operation can be divided into three stages —

(*a*) Fixing the fly in the vice
(*b*) Passing the pipette over the proboscis of the fly
(*c*) Adding the feeding fluid to the pipette

(*a*) The ideal method would be for each worker to carry out one step in the operation, but as this is not usually possible the best alternative is for one worker to prepare the flies in batches of about six at a time, that is to say, six flies are put each in a vice, then the dissecting microscope is brought into operation and the pipettes passed over the proboscis, and then the feeding fluid added to the six pipettes in one operation This was the method the present writer adopted, he found that he could feed about 12 flies in an hour on an average One or two flies are placed in an ordinary test-tube and a few drops of ether dropped on to the cotton plug, after a short interval the flies will become anæsthetized and when all movements have ceased they are thrown out on to a clean sheet of paper The legs of the fly, which are usually stretched out and lying together, and seized by the rubber-tipped forceps* in the right hand and with the vice in the left hand the wings of the fly are gripped in the extended position in the vice (Fig 2, D), the fly is held in such a way that it has free movement of its legs but the thorax is immobilized

* These are made by placing two triangular pieces, 0·5 cm sides, cut from a sheet of rubber (old motor tyre, for example) over the tips of a pair of sharp-pointed dissecting forceps

(Plate XLV, figs 1 and 2) The vice is then put into the glass tube ready for the next stage of the operation The important points in this stage are to get the fly properly anæsthetized (it is difficult to over-anæsthetize with ether), and to avoid damaging the flies' legs by exerting the slightest tension on them, it is a good precaution to chalk the rubber tips of the forceps to prevent the legs sticking to them Any active movement on the part of the fly before it is properly fixed will almost inevitably lead to some damage being done, but once it is fixed in the vice and the legs released, it seldom damages itself This step is best carried out with the aid of binocular magnifying glasses This is the most important and probably the most difficult part of the operation, as, if the fly is properly fixed and the apparatus is working freely, the second step is very simple

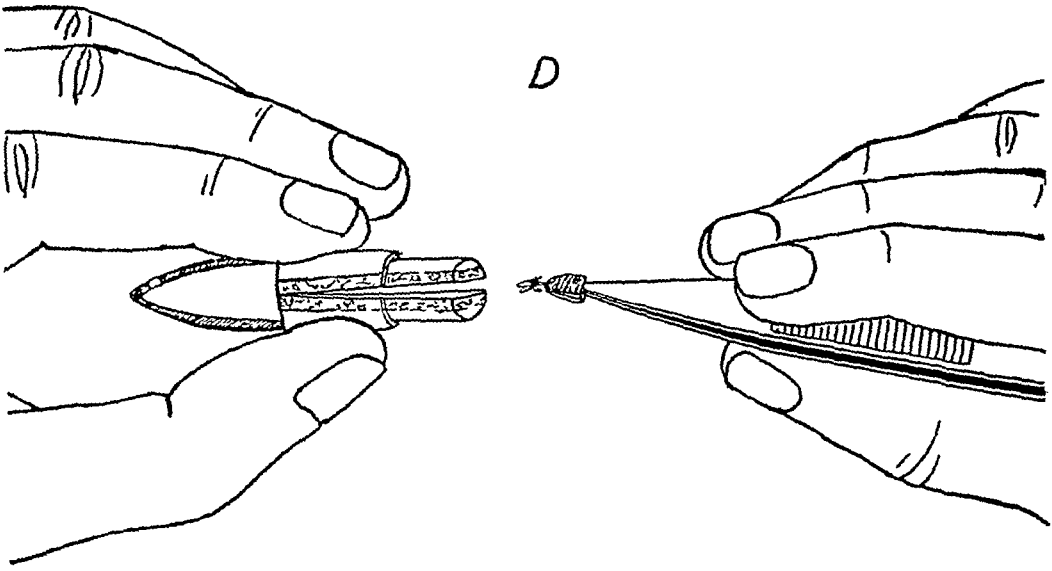

Fig 2

D The anæsthetized fly being placed in the vice by the aid of rubber-tipped forceps

(*b*) This step *should be done under a dissecting microscope* If the proboscis is pointing in the direction of the long axis of the feeding pipette it is often possible to pass the pipette over it in one movement, but, if the head is too much flexed for this to be done as is usually the case, the head should be extended by a stroking movement with the point of the pipette when the proboscis will often slip into the opening If the proboscis does not slip in easily the point of the pipette should be examined to see that it has not become blocked Usually both the stilettes and the labia pass into the pipette When in position the labia are apparently drawn up slightly by the fly We have not found it necessary as stated by Hertig and Hertig (1929), or even advantageous, to have the opening in the pipette so small that the labia are prevented from entering When the proboscis is once in it does not slip out very

readily, and if possible it should be moved so that the pipette forms an angle of 60° with the long axis of the body of the fly The apparatus is now fixed into a tray of plasticine ready for the addition of the feeding fluid The head of the fly with the proboscis in position inside the pipette is shown in Plate XLV, fig 3

(*c*) If this is not done immediately the apparatus should be examined to see that the fly has not meanwhile slipped its proboscis out of the feeding pipette as, once the fluid is added, it is almost impossible to get it into position again The fine tube covering the pipette is first removed by inverting the apparatus and allowing it to fall off, this is much safer than taking it off with the fingers as the slightest pressure on the feeding pipette may move it and allow the proboscis to escape The apparatus is then placed back in the plasticine tray in a vertical position With the aid of an ordinary glass pipette and rubber-teat a drop of the feeding fluid is allowed to come in contact with the open end of the feeding pipette, capillary attraction aided by gravity will cause the feeding pipette to fill throughout its length Directly the fluid reaches its proboscis the fly begins to feed If the fluid is coloured it can be seen passing along the œsophagus through the semi-transparent thorax of the fly As the fluid passes into the midgut the whole abdomen swells, in some instances becoming almost spherical Out of each batch a few flies will take a full meal in less than one minute, others will only take a partial meal, however long they are left, and yet others will not feed at all In some instances slight adjustment of the pipette will cause the fly, which was previously not feeding to feed, but the usual experience is that if a fly does not feed immediately it will not feed at all

The size of the opening and the angle at which the pipette is adjusted are undoubtedly important points, but the success of the operation does not depend entirely on these, and there appear to be some flies which will not feed however well the pipette is adjusted

Males as well as females can be fed, but the abdomens of the former never become tumid and spherical, when distended with blood they assume a sausage shape

After the fly has fed, the pipette is withdrawn, the vice removed from the glass tube, and the fly dropped into a test-tube The pipette should not be left in position longer than necessary, as evaporation of the feeding fluid may cause the pipette to adhere to the proboscis and the head of the fly, so that the latter is pulled off when the pipette is withdrawn

The pipette should be washed out immediately under a running tap with the narrow opening held towards the stream of water It should then be placed in hydrochloric acid which should be heated gently, then into absolute alcohol and boiled, and finally boiled thoroughly in 2 or 3 changes of distilled water If these precautions are not taken the lumen is liable to become blocked

Sterilizing—In order to ensure sterility, the whole apparatus filled up ready for use with the vice removed and replaced by a cotton plug is placed

in a small test-tube and sterilized in a hot air steriliser at about 120°C for ten minutes Care should be taken *not* to heat it to a higher degree than this or the cork ball will be spoilt

Preliminary feeding experiments—At first for the purpose of mastering the technique the writer used a dilute solution of eosin as the feeding fluid, later, material obtained by spleen puncture (from kala-azar patients) to which washed human blood corpuscles had been added, a number of successful feeds were carried out with this fluid, and in a number of instances the dissected sandfly showed flagellate forms of leishmania in the midgut, but on the whole the mortality rate was high and the infection rate was low It was not certain what was the cause of this high mortality, at first it was attributed to contamination of the material by oil in *which the spleen puncture syringe* was sterilized, but even when dry oil-free syringes were used the mortality was still high, and may have been due to imperfect fragmentation of the spleen pulp, or to contamination of the material whilst it was being ground up

Results of feeding experiments with culture material—The experiments summarized below were all done with leishmania cultures, *L donovani* cultures fed to *Phlebotomus argentipes* —

	Males	Females	Total
Number put up for feeding	102	254	356
Number feeding, fully	9	104	113
„ „ partially	71	118	189
TOTAL	80	222	302

With other species of sandfly and other culture material the feeding results were much the same In the interests of brevity these summaries have not been included

The length of time of survival after initial feed—In some instances when it was anticipated that the flies would die overnight they were killed and dissected, but the great majority were allowed to die naturally and were dissected very soon after death This table must not, therefore, be considered to indicate the natural survival rate

	Males	Females	Total
Total number	80	222	302
Survived 24 hours	47	156	203
„ 48 „	29	87	116
„ 72 „	12	50	62
„ 96 „	2	22	24
„ 120 „		15	15
„ 144 „		7	7

Development of leishmania in the sandfly—At first only the condensation fluid from NNN tubes was used for feeding the flies, but as it was found that development did not occur in the large majority of instances washed blood

corpuscles were added and mixed with the condensation fluid The figures quoted below are based on the dissections of flies that had survived at least 48 hours, as the presence of flagellates at an earlier date was not necessarily evidence that multiplication had occurred

L donovani *in* P argentipes—Of 25 flies dissected 48 hours or more after the initial feed of condensation fluid alone, only one contained flagellates, whereas of 73 flies given condensation fluid *plus* washed corpuscles 47 contained flagellates Both males and females were infected

L tropica *in* P argentipes—Of 12 flies fed on condensation fluid, none contained any flagellates, whereas of 27 fed on a flagellate culture *plus* washed corpuscles, 17 contained flagellates

L donovani *in* P minutus (babu)—Of 24 flies fed on flagellate culture *plus* washed corpuscles, none showed any parasites

In addition a few *P papatasii* were fed upon cultures of *L donovani plus* washed corpuscles, in no case were any flagellates observed, but the experiments were too few for the observation to be considered of any importance

No particular difference was noted in the degree of infection with the two different species of flagellate, it varied considerably in different individual flies There was usually a heavy infection of the proventricular end of the midgut, appearing to extend into the œsophagus, but in no instance was the diverticulum infected In a few instances in which sections were cut the œsophagus was seen to be blocked with a plug of flagellates, but in no case was infection of the pharynx noted, this is almost certainly because the flies did not live long enough for the infection to travel so far forward

Conclusions

The only conclusion that can be drawn from these few preliminary observations (which are reported now because the writer is going on leave) are —

That in the absence of red blood corpuscles, little proliferation of either *L donovani* or *L tropica* occurs in the sandfly *P argentipes*

That both these species of leishmania develop equally well in this sandfly in the presence of red blood cells

That in the sandfly *P minutus* (*babu*), *L donovani* does not proliferate or, at least, does not proliferate so readily as in *P argentipes*

My thanks are due to my assistants, Dr C R Das Gupta and Mr S Mukerjee, for assistance in breeding and dissecting the flies which were used in these experiments, also to Mr Woodhouse of the Survey of India for many valuable suggestions regarding the design of the apparatus and for preparing the cork and wood vices, the cork balls, and the gauge for measuring the pipettes

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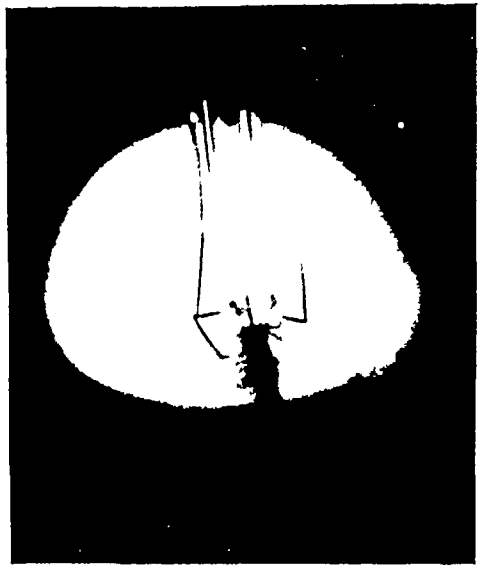

Fig 3

Explanation of Plate XLV

Fig 1 A sandfly, *P argentipes*, in the vice with the pipette in position, ready for the addition of the feeding fluid to be added In this piece of apparatus a window has been cut in the connecting tube in order to facilitate the taking of the photograph The proboscis of the fly can be seen quite clearly lying within the lumen of the feeding pipette The excessive number of legs this fly has must be due to movement when the photograph was being taken

„ 2 A sandfly, *P minutus*, in the vice with the pipette in position

„ 3 A 'close-up' of the head of a sandfly, *P argentipes*, with the proboscis lying in the lumen of the feeding pipette

SEROLOGICAL DIAGNOSIS OF SYPHILIS IN LEPERS

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EVER since the discovery of complement FIXATION Reaction by Bordet and Gengou (1901) and its application to the diagnosis of syphilis by Wassermann and his colleagues (1907), an enormous amount of work has been done upon it, with the general result of confirming its value as an aid to diagnosis and a control in the treatment of syphilis, yaws and leprosy The difficulty of standardizing the reagents on the one hand, and the technical difficulties associated with actually conducting it on the other, have been such that many attempts have been made to replace it by simpler methods, in which the interacting substances are reduced to 'antigen,' 'serum' and 'electrolyte' Numerous tests of this kind have been elaborated such as the flocculation reactions of Meinicke, (1917, 1918), Sachs-Georgi (1918), Vernes (1920), Dreyer and Ward (1921) and several others

Recent literature has contained many articles on the value of a new precipitation reaction devised by R L Kahn (1925), and it appears to be widely used in many of the American clinics The correlation of Kahn test with the classical Wassermann reaction and its many modifications in the case of syphilis, has been estimated by various workers A correlation as high as 97 6 per cent has been reached by Kahn (1924) using his 'standard' technique

Influenced by such encouraging reports, a series of tests were carried out in duplicate—both the Kahn and the Wassermann techniques being employed —with the object of determining whether from a purely qualitative point of view there is any advantage to be gained from the use of Kahn precipitation reaction as a method of diagnosing syphilis in lepers

KAHN'S QUALITATIVE PROCEDURE

As far as simplicity of procedure is concerned one could not wish for a better test than Kahn's latest modification of his test Antigen (consisting of an alcoholic extract of beef-heart muscle enriched with cholesterin) and 'Serum,' are mixed in varying proportions and vigorously shaken After a short period of incubation, which is desirable but not essential, saline is added and readings taken of the degree of precipitation produced

Test proper

Apparatus —

1 Agglutination tubes—7 5 cm in length and 1 c c diameter
2 'Standard' tubes—for mixing of antigen and saline, 5 5 cm in length, 1 5 cm diameter
3 Pipettes—1 c c graduated in 0 01 c c
2 c c „ in 0 001 c c
10 c c „ in 0 1 c c
4 Special racks—Kahn himself uses the agglutination type of racks but provided with three rows of holes instead of one Where only a dozen tests or so have to be carried out, ordinary agglutination racks would serve the purpose
5 A mechanical shaker is desirable but not necessary

The actual test consists of adding 0 15 c c of patients serum (inactivated for half an hour at 56°C) to three agglutination tubes containing 0 05 c c, 0 025 c c and 0 0125 c c of antigen-saline mixture which is prepared as follows —

About 1 c c of cholesterinized antigen is mixed with 1 c c of saline (the exact amount is determined by a special titration) as rapidly as possible and the mixture poured back and forth six times, and allowed to stand for ten minutes before using it in the test When ready, the requisite amount is added to each of the three tubes

The tubes are then shaken preferably in a shaking machine for two to three minutes and incubated in a water-bath at 37°C for 15 minutes Finally 0 5 c c of saline is added to each tube and results recorded within 5 minutes on the basis of ±, +, ++, +++ and ++++, depending on the degree of precipitation The final reading of the test is the 'average' of the degree of precipitation in the three tubes, i e , if the total number of pluses in the three tubes is, say, 12 (or 11) the reaction is ++++, if 9 (or 8) the reaction is +++, if 6 (or 7) the reaction is ++ and so on A reaction of ++ or more is interpreted as positive, a + or ± reaction as doubtful

Wassermann reaction employed for correlating purposes was conducted according to 'Method Number Four' as described in the Medical Research Committee Report (1918) the only difference being that the sera were tested up to 8 minimum hæmolytic doses of complement instead of the usual five

Source of material

All the specimens of blood examined were taken from patients admitted to the Lepei Asylum, Subathu (Simla Hills) A total of one hundred cases was examined

Results with Wassermann technique

1 Total number of *positive* cases by Wassermann reaction 27
(Details—Sixteen cases deviated 8 M H D of complement completely, five cases 5 M H D and six cases 3 M H D completely)

A 'single plus reaction' is considered to be of sufficiently diagnostic importance to label the case as positive Complete hæmolysis in all the tubes is interpreted as negative and partial hæmolysis in the front row tubes, as doubtful

2 Total number of *negative* cases by Wassermann reaction 64

3 Total number of cases deviating 3 M H D of complement incompletely 9

Results with Kahn reaction

(*a*) Total number of *positive* cases by Kahn reaction 32
(Details—Nineteen cases showed a quadruple plus reaction, nine cases a treble plus and four cases a double plus)

(*b*) Total number of *negative* cases by Kahn reaction 58

(*c*) Total number of cases showing a single plus reaction 4

(*d*) Total number of cases showing a plus minus Kahn reaction 6

Table

Comparison of Wassermann and Kahn reactions

		Wassermann test	Kahn test
Negative		64	58
Doubtful		9	6
Positive	Single plus reaction	6	4
	Double plus reaction	5	4
	Treble plus reaction	16	9
	Quadruple plus reaction		19

Conclusions

The value of the precipitation reaction of Kahn was studied in specimens of blood from one hundred leprosy cases in comparison with the Wassermann test There was absolute agreement in 90 per cent of cases

2 The test is fairly simple and brief It compares more than favourably with the Wassermann reaction as judged from a qualitative point of view It is of special help in cases where rapid results are desired but it needs a little practice to read the extents of precipitation correctly Wassermann results are comparatively much more easy to read With Kahn's reaction, however, in a certain number of cases it is extremely difficult to differentiate between the negative, doubtful and a single plus reaction

3 The question arises whether leprosy *per se* gives a positive Wassermann reaction The modern concensus of opinion (Lloyd, 1927) is that unless syphilitic infection is present in addition, a positive reaction is an exception rather than the rule

We are highly indebted to Mr G H Watson, Superintendent, Leper Asylum, Subathu, for his very kindly supplying us with sera of leprosy patients, and to Major J A Sinton, V C, O B E, I M S, for his kind advice and criticism

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EXPERIMENTS IN DOSAGE OF CARBOLIZED ANTI-RABIC VACCINE

BY

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THE present series of experiments was designed to test the immunizing value of carbolized anti-rabic vaccine in relation to the dosage of fixed virus brain administered

The vaccine used was the standard Indian one per cent fixed virus brain emulsion with 0 5 per cent carbolic acid, altered in certain cases by filtration, preliminary ether extraction, or other treatment Rabbits were used for the tests and the immunizing course given consisted in each case of fourteen daily injections of the dose stated The doses given were the equivalent human doses of the standard Indian course in proportion to weight or multiples of these amounts The test infection after the completion of treatment was by corneal scarification and the application of a thick emulsion of first passage street virus

EXPERIMENT No 1 (TABLE I)

Forty rabbits were used divided into four groups of ten each

The following immunizing treatments were given —

Group No 1 Controls Not immunized

„ No 2 Equivalent human dose of one per cent carbolized vaccine

„ No 3 Five times above dose

„ No 4 Ten times above dose

Infection by corneal scarification was carried out eleven days after the completion of treatment

The results are summarized as follows —

Group	Original number	Remaining for test	Died of rabies	Died otherwise	Survived
No 1	10	10	9	1	
No 2	10	10	5		5
No 3	10	9	4	1	4
No 4	10	8		2	6

Of the two rabbits in Group No 4 which are shown as 'died otherwise,' both died on the 58th day after infection One showed no Negri bodies and sub-passage was negative The brain of the other was decomposed, the rabbit having died overnight

The results show an increased protection in the group receiving the highest dosage

EXPERIMENT No 2 (TABLE II)

Four groups of ten rabbits each were used in this series, the vaccine employed in the case of Group Nos 3 and 4 being modified as stated The immunizing doses given were —

Group No 1 Controls Not immunized

„ No 2 Five times the equivalent human dose of one per cent carbolized vaccine

„ No 3 The same dose of the same vaccine after filtration through Chardin filter paper

„ No 4 Five times the equivalent human dose of a vaccine prepared by grinding fixed virus brain with sterile sand, treating with nine per cent saline, diluting to 0 9 per cent saline and filtering through Chardin filter paper The emulsion before filtration contained one per cent of original brain substance

The method of preparing the vaccine used for Group No 4 was based on the procedure adopted by Hindle in regard to emulsions of spleen of Macacus monkeys infected with yellow fever virus This was tried with the object of dissociating the virus from the brain substance

The infecting dose was given ten days after the completion of treatment The results are summarized as follows —

Group	Original number	Remaining for test	Died of rabies	Died otherwise	Survived
No 1	10	10	10		
No 2	10	10	4	1	5
No 3	10	10	8		2
No 4	10	10	9		1

The rabbit in Group No 2 shown as 'died otherwise' died 45 days after infection Sub-passage was negative

The total solids in the vaccines used when dried at 105°C gave the following percentages —

Group No 2	vaccine	1 06 per cent
„ No 3	„	0 93 „ „
„ No 4	„	0 96 „ „

The vaccines used for these groups contained 0 85 per cent, 0 85 per cent and 0 875 per cent NaCl respectively, the deduction of which leaves the following percentages of dried brain substance in each —

Group No 2	vaccine	0 21 per cent
„ No 3	„	0 08 „ „
„ No 4	„	0 085 „ „

We have found that the average percentage of dried solids in rabbits brain is approximately 21 per cent so that these vaccines would correspond to one per cent, 0 381 per cent and 0 405 per cent of original brain substance respectively

The results of the experiment show that the removal of brain substance by filtration greatly reduces the immunizing value of the vaccine The special treatment of the vaccine used for Group No 4 did not result in any increase of antigenic value

Experiment No 3 (Table III)

This experiment was designed to show the effect of high dosage and also of preliminary ether treatment of fixed virus brain before preparation of the carbolized vaccine

Fifty rabbits in five groups of ten each received the following immunizing treatments —

Group No 1 Controls Not immunized

„ No 2 Equivalent human dose of one per cent carbolized vaccine

„ No 3 Five times above dose

„ No 4 Equivalent human dose of a vaccine prepared from the same fixed virus brain as used for Group Nos 2 and 3 The brain substance used was broken into small portions and immersed in ether for 72 hours A carbolized vaccine was then prepared in the usual manner containing the equivalent of one per cent original brain

„ No 5 Five times the equivalent human dose of the same vaccine as used for Group No 4

Infection by corneal scarification was carried out ten days after completion of treatment

The results are summarized as follows —

Group	Original number	Remaining for test	Died of rabies	Died otherwise	Survived
No 1	10	10	10		
No 2	10	9	8		1
No 3	10	10	5		5
No 4	10	10	10		
No 5	10	10	3	1	6

The rabbit shown as 'died otherwise' died seven days after infection and sub-passage was negative

In this series no protection was shown by the use of the equivalent human dose of one per cent carbolized vaccine prepared with or without preliminary ether treatment With five times the equivalent human dose a 50 per cent protection was obtained with the ordinary vaccine, and a somewhat higher protection with the ether-treated vaccine The difference is probably not significant

The removal of ether-soluble material did not lessen the antigenic value of the vaccine

Experiment No 4

In this experiment three groups of ten rabbits each received corneal infection with first passage street virus and immunization was commenced on the following day The groups were —

Group No 1 Controls
„ No 2 Equivalent human dose of one per cent carbolized vaccine
„ No 3 Five times above dose

There were no survivors This corresponds with Cunningham and Malone's finding that rabbits are unsuitable for post-infection immunization experiments

Comment

This series of experiments which was limited to 160 rabbits on account of the shortage of animals gives certain indications with regard to the value of some of the factors concerned in anti-rabic immunization with carbolized virus Cunningham and Malone (1930) have found that, in the proportionate dosage used, etherized vaccine given by a modification of Ahrisatos' method was superior to the carbolized vaccine, but the total amount of fixed virus brain given by the Ahrisatos' method was several times greater than the dosage of carbolized vaccine in their experiments In the present series increased dosage of carbolized vaccine has been tried and, although the total figures are small, there is a definite indication that higher dosage than that equivalent to the

standard immunizing course used for human beings in India will result in a higher degree of protection being obtained Experiments Nos 2 and 3 show that the removal of brain substance from the vaccine by filtration through filter paper results in a great reduction in antigenic value The vaccines used for these experiments had been stored in the refrigerator for five weeks before use Attempts to break up the brain substance so as to obtain a higher proportion of antigenic substances after filtration did not appear to have been successful The removal of ether-soluble material by preliminary immersion in ether for 72 hours did not appear to affect the immunizing value of the vaccine Post-infection immunization experiments with rabbits were unsuccessful, all animals dying This confirms the finding of Cunningham and Malone at Kasauli that these animals are unsuitable for this type of experiment It should be noted that, in some instances, protection of rabbits against post-immunization infection by the method used was obtained with the equivalent human dosage of carbolized vaccine, which according to the above quoted authors has never been the experience at Kasauli In the present series, and in previous work carried out in Rangoon (Gloster, Taylor and Menon, 1926), this dosage has resulted in a protection varying from 80 per cent to nil with 100 per cent mortality of controls These results suggest that there is a considerable difference in the virulence of street viruses obtained even in one locality Cunningham and Malone have expressed their opinion that differences in virulence exist in different parts of India and support this by reference to the results of treatment at various centres Our experimental results would suggest a low virulence as being the rule in Burma and the statistical results of treatment in Rangoon as compared with institutes in India support this view

Conclusions

The short series of experiments detailed shows that —

(1) a dosage of carbolized vaccine considerably in excess of the equivalent human dose of the standard treatment results in improvement in the protection obtained in the case of rabbits,

(2) the removal of brain substance from the vaccine by filtration greatly reduces its immunizing value,

(3) the preliminary extraction of ether-soluble material from the fixed virus brain does not affect the value of the vaccine

Indications of difference of virulence of street virus strains shown by protection experiments are commented on

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TABLE I

Group	Rabbit number	Weight in grammes	Protective treatment	Infecting dose	Result	Days to death	REMARKS
No 1	1	800	Nil	Corneal scarification with first passage street virus 11 days after completion of treatment	Died	58	Brain decomposed
	2	600			Do	18	Negri bodies +
	3	900			Do	19	Do
	4	600			Do	23	Do
	5	1,000			Do	21	Do
	6	600			Do	23	Do
	7	800			Do	20	Do
	8	700			Do	22	Do
	9	800			Do	25	Do
	10	750			Do	19	Do
No 2	11	800	Equivalent human dose for weight of one per cent carbolized vaccine 14 daily injections	Do	Do	10	Do
	12	700			Survived		
	13	700			Do		
	14	800			Do		
	15	800			Died	22	Negri bodies +
	16	800			Do	28	Do
	17	800			Survived		
	18	800			Died	33	Negri bodies +
	19	600			Do	30	Do
	20	700			Survived		

No 3	21	1,100	Five times equivalent human dose for weight of one per cent carbolized vaccine 14 daily injections	Do	Died	26	Negri bodies +
	22	1,000			Do	28	Do
	23	850			Survived		
	24	800			Died	59	Negri bodies — Sub-passage —
	25	600			Survived		
	26	950			Do		
	27	950			Do		
	28	900			Died	23	Negri bodies +
	29	900			Do	23	Do
	30	600			Do		Died before test
No 4	31	600	Ten times equivalent dose for weight of one per cent carbolized vaccine 14 days injection	Do	Do		Do
	32	800			Do	58	Brain decomposed
	33	800			Survived		
	34	1,000			Do		
	35	800			Do		
	36	700			Do		
	37	700			Died		Died before test
	38	600			Survived		
	39	1,000			Died	58	Negri bodies — Sub-passage —
	40	800			Survived		

TABLE II

Group	Rabbit number	Weight in grammes	Protective treatment	Infecting dose	Result	Days to death	Remarks
No 1	1	1,050	Nil	Corneal scarification with first passage street virus 10 days after completion of treatment	Died	19	Negri bodies +
	2	1,100			Do	20	Do
	3	1,100			Do	18	Do
	4	1,150			Do	18	Do
	5	1,200			Do	25	Do
	6	1,300			Do	18	Do
	7	1,300			Do	18	Do
	8	1,350			Do	17	Do
	9	1,500			Do	21	Do
	10	1,000			Do	20	Do
No 2	11	1,050	Five times the equivalent human dose of one per cent carbolized vaccine 14 daily injections	Do	Do	15	Sub-passage — Negative
	12	1,100			Do	25	Negri bodies +
	13	1,100			Do	28	Do
	14	1,150			Survived		
	15	1,200			Do		
	16	1,300			Do		
	17	1,300			Do		
	18	1,350			Died	22	Negri bodies +
	19	1,400			Do	22	Do
	20	1,250			Survived		

No 3	21	1,100			Died	28	Negri bodies +
	22	1,100			Do	26	Do
	23	1,150			Do	17	Do
	24	1,200	Five times the equivalent human dose of the same one per cent carbolized vaccine after filtration through Chardin filter paper 14 daily injections	Do	Survived		
	25	1,200			Died	18	Negri bodies +
	26	1,300			Survived		
	27	1,300			Died	18	Negri bodies +
	28	1,350			Do	21	Do
	29	1,400			Do	19	Do
	30	1,100			Do	19	Do
No 4	31	1,100			Do	21	Do
	32	1,150			Do	24	Do
	33	1,200	Five times the equivalent human dose of a one per cent carbolized vaccine made by grinding fixed virus brain with sand, treating with nine per cent saline, dilution to 0·9 per cent saline and filtering 14 daily injections	Do	Do	24	Do
	34	1,200			Do	19	Do
	35	1,300			Do	22	Do
	36	1,300			Survived		
	37	1,350			Died	23	Negri bodies +
	38	1,400			Do	21	Do
	39	1,400			Do	17	Do
	40	1,250			Do	28	Do

TABLE III

Group	Rabbit number	Weight in grammes	Protective treatment	Infecting dose	Result	Days to death	Remarks
No 1	1	1,080	Nil	Corneal scarification with fixed passage street virus 10 days after completion of treatment	Died	22	Negri bodies +
	2	1,060			Do	17	Do
	3	1,180			Do	19	Do
	4	1,180			Do	20	Do
	5	1,160			Do	23	Do
	6	1,230			Do	19	Do
	7	1,230			Do	18	Do
	8	1,250			Do	18	Do
	9	1,430			Do	21	Do
	10	1,030			Do	22	Do
No 2	11	1,080	Equivalent human dose of one per cent carbolized vaccine 14 daily injections	Do	Survived		
	12	1,080			Died	30	Negri +
	13	1,080			Do	18	Do
	14	1,080			Do	28	Do
	15	1,140			Do	23	Do
	16	1,180			Do	20	Do
	17	1,180			Do	28	Do
	18	1,150			Do		Died before infection
	19	1,450			Do	23	Negri +
	20	1,150			Do	18	Do

No 3	21	1,080	Five times equivalent human dose of one per cent carbolized vaccine 14 daily injections	Do	Do	20	Do
	22	1,100			Do	34	Do
	23	1,100			Survived		
	24	1,180			Died	23	Negri +
	25	1,130			Survived		
	26	1,200			Died	21	Negri +
	27	1,280			Survived		
	28	1,280			Do		
	29	1,600			Died	26	Negri +
	30	1,100			Survived		
No 4	31	1,050	Equivalent human dose of a vaccine prepared from same fixed virus brain Brain immersed in ether for 72 hours and carbolized vaccine containing one per cent original brain made 14 daily injections	Do	Died	17	Negri +
	32	1,000			Do	21	Do
	33	1,100			Do	23	Do
	34	1,130			Do	18	Do
	35	1,180			Do	20	Do
	36	1,130			Do	21	Do
	37	1,200			Do	27	Do
	38	1,250			Do	29	Do
	39	1,380			Do	23	Do
	40	1,000			Do	20	Do

TABLE III—*concld*

Group	Rabbit number	Weight in grammes	Protective treatment	Infecting dose	Result	Days to death	REMARKS
No 5	41	1,000			Survived		
	42	1,000			Died	7	Negri bodies — Sub-passage —
	43	1,080			Survived		
	44	1,050	Five times equivalent human dose of same vaccine as used for Group No 4 14 daily injections	Do	Died	25	Negri +
	45	1,180			Survived		
	46	1,180			Died	26	Negri +
	47	1,280			Survived		
	48	1,280			Died	33	Negri +
	49	1,380			Survived		
	50	1,000			Do		

A NOTE ON *CERCARIA ANOMALA* RAO

BY

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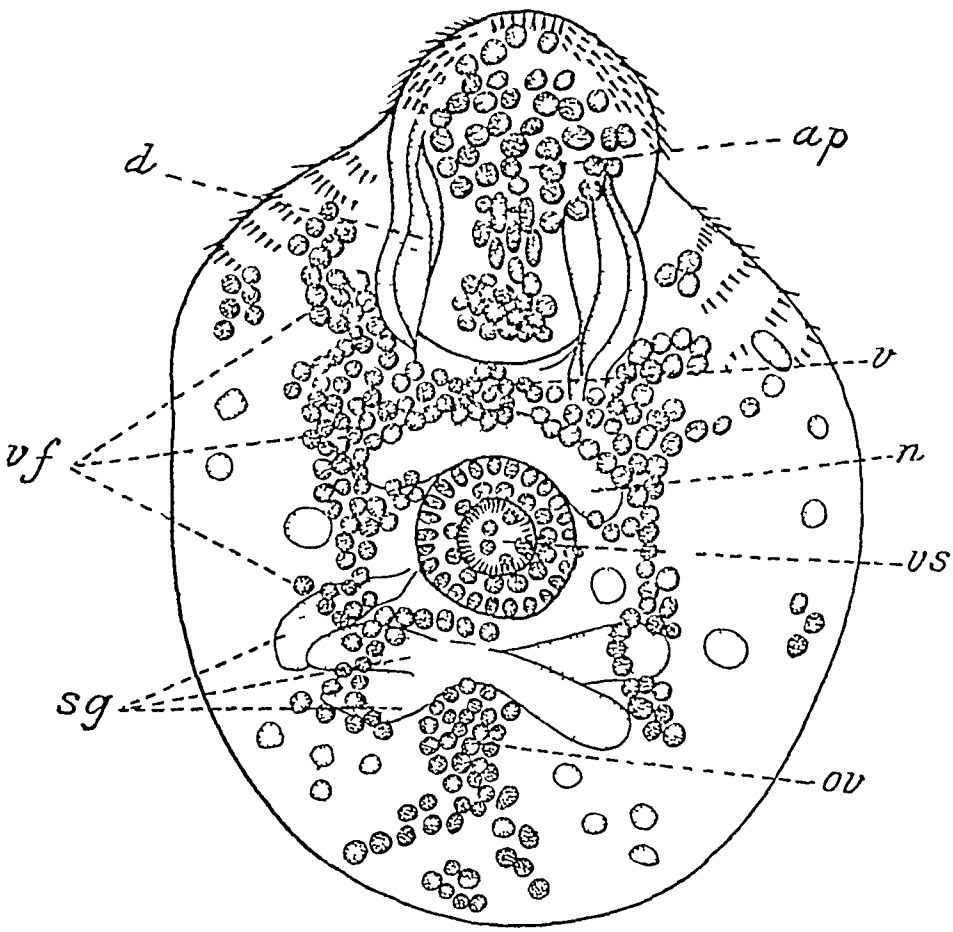
Cercaria anomala was described by Rao (1929) in a paper published in the Scientific Papers of the Civil Veterinary Department, Madras. There is no doubt that Rao has discovered a hitherto unknown species of cercaria, and, moreover, one that presents several characters of peculiar interest. Mr. Rao has been kind enough to send to me for examination stained and mounted specimens of this species, part of the material on which his account is based, and a study of this material has enabled me to confirm the original description and to add a few details.

Rao has described the rows of minute spines on the protrusible penetrating organ, but in addition to these there are also several rows of very minute spines on the anterior region of the body, as shown in the Text-figure below.

In his account of the genital system Rao only mentions the small though very distinct mass of cells situated between the acetabulum and the anterior wall of the excretory bladder, in addition to this mass of small cells there can be seen in the stained specimens a round mass of small but deeply stained cells situated in the middle line between the anterior margin of the acetabulum and the posterior end of the penetrating organ. This latter mass probably represents the rudiment of the genital aperture. Extending outwards from the middle line and situated on either side of the body from the level of the penetrating organ in front to the level of the genital rudiment and the anterior margin of the excretory bladder is a somewhat diffuse and irregular collection of cells that may represent the rudiment of the vitelline glands. If this be so, the structure of the complete genital system in this species would appear to be very similar in its general type to that described by Platt (1919) in *Cercaria fusca*, one of the cercariæ belonging to the Cystocercous group in which the tail-stem is enormously dilated.

At first sight one would, on account of the peculiarities of the tail-stem and furcal rami, be inclined to refer *Cercaria anomala* Rao to the 'Mirabilis' group of the Cystocercous cercariæ (*vide* Sewell, 1922, p. 295). The various species that have been grouped together in this subdivision can be separated into two series according to the relationships of the distome body and the tail-stem. In the first division may be placed those species in which the distome body is enclosed within the anterior part of the tail-stem, to this division belong



Text-figure—*Cercaria anomala* Rao

ap Anterior penetrating organ, *d* Salivary ducts, *n* Central nervous mass, *ov* Ovary, *sg* Salivary gland cells, *v* Vagina, *vf* Vitelline follicles, *vs* Acetabulum

Cercaria mirabilis Braun (1891), *Cercaria wrightii* Ward (1917), *Cercaria anchoroides* Ward (1917), and *Cercaria macrostoma* Faust (1918).

In the second division the tail-stem is greatly dilated but the distome body is not included within the anterior region of it and projects freely in front of it, as in the normal form of the cercariæ. To this division, belong *Cercaria brookoveri* Faust (1918), *Cercaria stephanocauda* Faust (1921*a*), *Cercaria pekinensis* Faust (1921*b*) and *Cercaria fusca* Platt (1919).

It is to this latter division that the present species would, if we were to judge by the character of its tail-stem and the arrangement of its genital system, appear to approximate. In all these latter forms the cercariæ arise

from sac-like or elongate sporocysts and possess large and distended tails that terminate in a pair of short furcal rami, while anteriorly there is either a definite collar-like expansion of the tail-stem or a series of transverse ridges, constituting a collar-region, the caudal excretory canal is wide and after traversing the whole length of the stem divides into two branches that enter the rami and in the case of *Cercaria pekinensis* Faust, *Cercaria fusca* Pratt, and *Cercaria stephanocauda* Faust, and probably *Cercaria brookoveri* Faust also, terminate through small apertures at the extreme tip In all these respects *Cercaria anomala* Rao shows agreement, but it differs in other important characters

All the above mentioned members of the second group of the Cystocercous cercariæ agree in the possession of a definite oral sucker, a well-developed pharynx and large intestinal cæca that extend back to the posterior end of the body In two of the species the excretory system has been worked out and it is probable that all true members of the group possess a system like that of *Cercaria pekinensis* Faust in which the main excretory tube passes forwards on either side of the body, receiving accessory tubules on its way, as far as the level of the oral sucker and then turns back and runs through the body again to terminate in the bladder This type of excretory system is very different from that found in *Cercaria anomala* Rao To which family of the Trematodes the adults of this group of the Cystocercous cercariæ belong is not certainly known, Faust (1918, p 152) has stated that the genitalia in one of the species examined, *Cercaria macrostoma*, 'quite definitely relate the group to the Allocreadiidæ' and Pratt has followed Faust's lead On the other hand Braun believes that in his species the structure reveals a relationship to the Furcocercous cercariæ and thus to the Schistosomatidæ or the Holostomes It must, in this connection, be borne in mind that the Cystocercous condition of the tail-stem may be an example of convergence

A somewhat similar enlargement of the tail-stem has been described in *Cercaria Tergestiæ haswelli*, that was originally described by Haswell (1902), who, however, did not give the form a name, and was subsequently named by Dollfus (1927), who also briefly describes and names a second form, *Cercaria kenti*, that had previously been recorded by Saville Kent, who, however, mistook it for the 'larva of an Echinorhynchus' Unfortunately, too little is known of the structure of these two forms to enable one to place them in any of the recognized groups of cercariæ or to found a new group for them Both are apparently larval stages in the life-history of a species of *Tergestia* (*vide* Odhner, 1911, p 529) and thus belong to the sub-family Steringophorinæ of the family Steringophoridæ

The internal structure of *Cercaria anomala* Rao differs in several fundamental particulars from any of the above-mentioned forms The anterior region is occupied, not by an oral sucker but by a definite penetrating organ, armed with a group of anteriorly directed spines, at or near the base of which the ducts of the penetrating gland cells open There is no pharynx and the

œsophagus terminates in a small bulbous dilatation anterior to the ventral sucker and is not continued back as intestinal cæca Finally, a study of the excretory system shows that it conforms to the type present in certain Furcocercous cercariæ With the exception of the cystic enlargement of the tail, the structure of this species agrees closely with that of certain of the Furcocercous cercariæ and particularly with those of the Schistosome series A very similar enlargement of the tail region has been recorded by Miller (1927) in certain other Furcocercous cercariæ, namely, *Cercaria bulbocauda* and *Cercaria absurda,* though in these two instances the cystic enlargement is confined to the posterior region and is not uniform throughout the whole length of the tail-stem A study of the excretory system of these two latter forms shows, however, that although they, like *Cercaria anomala* Rao, belong to the Furcocercous series, they must be placed in different divisions *Cercaria anomala* Rao clearly belongs to the apharyngeal brevifurcate distome group, whereas *Cercaria bulbocauda* Miller and *Cercaria absurda* Miller are pharyngeal longifurcate forms and are, in all probability, members of the Holostome series

It would thus appear that there are at least four groups of cercariæ in which this cystic enlargement of the tail-stem has been independently developed

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The following has been received from the War Office, London, dated the 5th September, 1930 —[Ed]

NORTH PERSIAN FORCES MEMORIAL MEDAL

Captain H W Mulligan, m b , Indian Medical Service, has been awarded the North Persian Forces Memorial Medal for the year 1929 for his paper 'Studies on the Reticulo-Endothelial System, with Special Reference to Malaria,' published in *The Indian Journal of Medical Research*, Vol XVI, No 4, April 1929, pp 1099–1119

This Medal is awarded annually for the best paper on Tropical Medicine or Hygiene published in any Journal during the preceding twelve months by a Medical Officer, of under twelve years' service, of the Royal Navy, Royal Army Medical Corps, Royal Air Force, Indian Medical Service, or of the Colonial Medical Service, provided the Memorial Committee consider that any of the papers published has attained a standard of merit justifying an award

A RAT-FLEA SURVEY OF THE MADRAS PRESIDENCY

BY

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REPORT NO VII

BERHAMPORE, VIZAGAPATAM AND BEZWADA, IN THE NORTHERN AND EASTERN PARTS OF THE PRESIDENCY

(The Report for Nellore in Report No IV published in the *Indian Journal of Medical Research* for April 1930 can with advantage be grouped with these)

(A) BERHAMPORE * (September-October 1929)

BERHAMPORE, a municipal town, is the headquarters of the Ganjam district, the northernmost district in the Madras Presidency It is situated on the main railway line from Madras to Calcutta The town with an extent of about six square miles is divided into three important areas intercepted by a series of large tanks which are used for agricultural purposes These are —

(1) Bapur forming the eastern third of the town, inhabited mainly by the more well-to-do classes, (2) Berhampore proper or old Berhampore, which forms the western half of the town and is inhabited by the older class of residents who are mostly Oriya agriculturists and weavers, and (3) Bijjapur, a small hamlet of shepherds and labourers which is more or less wedged in between Bapur and Berhampore

Population—The population according to the census of 1921 is 32,731

* This place was surveyed by Dr D S Mankikar

Housing and sanitation—The town is hemmed in on all sides by rice fields and vegetable gardens In Bijjapur and its suburbs one finds only mud huts with thatched roofs with lofts for storing corn and hay which thus afford ideal shelters for breeding rats In Bapur and Berhampore, however, there are better class houses though these, with few exceptions, do not by any means compare favourably with the former except in the matter of structural design The houses everywhere are dark and ill-ventilated The level of the subsoil water being very high, the houses usually are very damp There is no drainage system of any kind and sanitation is very poor

Climate and rainfall—The climate is generally hot and humid It was particularly damp at the time of the survey

The average annual rainfall is 50 inches

Period of survey–15th September to 6th October, 1929

Mean dry bulb temperature 8 A M 80 6° Fahr

„ wet bulb temperature 8 A M 78 8° Fahr

„ relative humidity 8 A M 94 0 per cent

„ saturation deficiency 8 A M 0 06 inches

Plague—There is no history of plague having occurred in any form in the town

Imports and exports—Paddy is the chief crop of the district and this and rice are exported in large quantities to Vizagapatam Green gram and horse gram come from the surrounding villages for export to the southern districts Gingelly seed is mainly exported to Rangoon

As regards imports, dhal, wheat, gram and peas come chiefly from Cawnpore and Nagpur which are plague-infected areas Miscellaneous articles come from Calcutta An additional source of infection is the return of labourers from Rangoon where temporary employment is usually obtained

Rodents—Details are given in Table I

Of the 397 rodents trapped during the survey, only five were *R norvegicus* (3 ♂ and 2 ♀) the rest were *R rattus* Both varieties, the white-bellied and the brown-bellied were found, the latter predominating–72 5 per cent The percentage of female rats was larger than that of the males From the table it will be seen that the density of rats was not only high in the bazaars but also in the residential suburbs of Bijjapur, viz, in Comapalli and Goyilandi which are almost exclusively inhabited by ryots The high rat density here can easily be explained by their huts being used more or less as granaries All the rats trapped were autopsied and spleen smears examined for the presence of *B pestis* All were negative

Fleas—Details are given in Table I

The general flea index varied from 3 6 to 5 68 The *astia* index was comparatively low in all localities and varied from 0 96 to 2 26 The *cheopis* index on the other hand was high and varied from 1 33 to 5 68 This maximum was reached in Comapalli and Goyilandi where no other rat fleas were obtained

TABLE I

Rodents and fleas–Berhampore (September-October 1929)

Locality	Number of traps laid	Number of rats trapped	Rat density (number of rats per 100 traps)	Total number of fleas	General flea index for *R rattus*	*X astia*	*Astia* index for *R Rattus*	*X cheopis*	*Cheopis* index for *R rattus*
Bijjapur bazaar	48	70	146	260	3·71	17	0·24	243	3·47
Bijjapur houses	72	31	43	142	4·58	39	1·25	103	3·32
Comapalli, Goyilandi, etc Ryots' huts	72	41	57	233	5·68			233	5·68
Bapur bazaar	72	53	74	275	5·18	67	1·26	208	3·92
Bapur houses	192	112	58	590	5·26	167	1·49	423	3·77
Berhampore bazaar	96	51	53	165	3·23	49	0·96	116	2·27
Berhampore houses	84	24	29	114	4·75	9	0·38	105	4·37
Rice mills	24	15	63	54	3·60	34	2·26	20	1·33
TOTAL	660	397	60	1,833	4·60	382	0·96	1,451	3·64

Percentage female rats	60·5	Percentage female *astia*	54·9
Percentage pregnant females to total females	16·6	Percentage *astia*	20·8
Average number of fœtuses	4·7	Percentage female *cheopis*	40·2
Replenishment rate for 100 rats per day	0·3	Percentage *cheopis*	79·2

SUMMARY

(1) Three hundred and ninety-seven rodents were trapped of which 392 were *R rattus* and 5 *R norvegicus*

(2) Out of 1,833 fleas examined, 382 or 20 8 per cent were *X astia* and 1,451 or 79 2 per cent were *X cheopis* Thus *X cheopis* is the predominating flea of Berhampore

(3) It is evident that the conditions as regards density of rats and of *cheopis* are suitable for the propagation of plague As the climatic conditions too are not unfavourable, we conclude that there is a grave danger of a severe plague epidemic should infection once be introduced How an epidemic has been escaped so far is not understood, unless *cheopis* infection was much less in the past In one way this previous absence of plague is an added danger since the rat population must be very susceptible to it We suggest to the local health authorities that it would be wise to take steps now to deal with the situation *before* plague arrives

(B) VIZAGAPATAM * (October-November 1929)

Vizagapatam, a municipal town, is the headquarters of the district of the same name It is two miles distant from Waltair on the main railway line running north-east from Madras to Calcutta A branch line connects Waltair with Vizagapatam station, but the town proper is almost equidistant from both It is a rising seaport and an extensive harbour is now under construction The town has an area of about six square miles and a population, according to the census of 1921, of 44,711

Sanitation and housing—The houses are of the usual south Indian pattern, mostly tiled, with the exception of a few terraced ones Most of the houses are in contiguity from side to side They have no ceilings and are dark and ill-ventilated Scattered here and there are groups, large and small, of mud huts with thatched roofs of palmyra leaves An attempt has been made at drainage, but as the ground is uneven sewage collects near houses Sanitation, except on the Beach Road, is very poor

Climate—The mean monthly temperature at 8 A M in 1929 varied from 73°F in January to 88°F in May The average annual rainfall is 40 inches

Period of survey–7th October to 8th November, 1929

Mean dry bulb temperature at 8 A M 78 8° Fahr

„ wet bulb temperature at 8 A M 73 4° Fahr

„ relative humidity at 8 A M 76 per cent

„ saturation deficiency at 8 A M 0 24 inches

Plague—There was one epidemic of plague in Vizagapatam during 1917-18 It broke out on 17th November, 1927, and continued in a rather severe form till the end of June 1918 The beginning of the epidemic was traced to an imported case, the first attack occurring in a passenger from Rangoon in Chengalraopettah

*This place was surveyed by Dr D S Mankikar

ward, whence it spread to other parts of the town There were altogether 500 deaths during the epidemic The town has been free from plague since then

Trade—Trade with Vizagapatam is both by sea and land At present ships lie at anchor some miles away and the cargo is landed with the help of lighters Quays for ships to moor alongside are, however, under construction When the harbour is completed, Vizagapatam will serve as a seaport not only for the East Coast but also for its hinterland—the United Provinces and Central Provinces—which export wheat, cotton and cotton seed Rice and paddy are mainly imported by rail from the Ganjam district Green gram comes from Kajipet in the Nizam's Dominions and gram and dhal from Bengal Myrobalans, groundnuts and gall-nuts from the surrounding districts, a small quantity of gunny bags from Calcutta and linseed and manganese ore from the Central Provinces are at present shipped to Europe from Vizagapatam Machinery and oils are the only imports by sea

Rodents—Details are given in Table II

For the purposes of the survey rats were trapped from the bazaar which is on the main road, and from the different wards, gram godowns and a small village in Waltair ward inhabited by ryots In all 435 *R rattus*, 7 bandicoots and 1 musk rat were trapped All three varieties of *R rattus* were present, the brown-bellied predominating—87 per cent

It will be seen that the bazaar shows a very high rat density of 100, next come the groundnut and gram godowns with densities of 88 and 83 respectively A high rat density of 75 also obtains in the huts of the ryots of the uplands village as these people usually store gram As usual the proportion of female rats was greater than that of males All the rats trapped were autopsied Their spleen smears were negative for *B pestis*

Fleas—Details are given in Table II

The bazaar and godowns showed as usual high general and specific flea indices In all localities both *astia* and *cheopis* were obtained, except in the small village at Waltair ward where only *cheopis* with a high index of 4 05 was obtained Amongst the fleas examined one female *X cheopis* with two spermathecæ was seen The percentage of female *astia* was found to be larger than that of the males, whereas the percentage of female *cheopis* was smaller than that of the males

Some interesting results were obtained at godowns storing rice and other grains compared with those storing groundnuts, myrobalans, etc An attempt was made to trap loose fleas from these godowns Rats were anæsthetized and all fleas from them removed by carefully searching their fur When they were thus completely 'de-fleaed,' they were transferred in pairs to traps previously washed and dried in the sun and at once covered with cloth bags These covered traps were taken to the godowns in the evenings, the covers removed and the entrances to the trap blocked to prevent the ingress of other rats The traps were brought to the laboratory in the usual way the next morning and fleas collected and examined Table III summarizes and compares the rat

TABLE II

Rodents and fleas–Vizagapatam (October-November 1929)

Locality	Number of traps laid	Number of rats trapped	Rat density (number of rats per 100 traps)	Total fleas	General flea index for *R rattus*	*X astia*	*Astia* index for *R rattus*	*X cheopis*	*Cheopis* index for *R rattus*
Bazaar	92	92	100	505	5·48	140	1·52	365	3·96
Residential areas	631	251	40	1,344	4·63	583	1·52	761	3·11
Groundnut godowns	25	22	88	49	2·22	32	1·45	17	0·77
Rice and grain godowns	72	60	83	457	7·61	149	2·48	308	5·13
Uplands village-ryots' huts	24	18	75	53	4·05			53	4·05
TOTAL	844	443	53	2,408	5·03	901	1·58	1,501	3·45

Percentage female rats	63·9	Percentage female *astia*	54·5
Percentage pregnant females to total females	17·7	Percentage *astia*	37·5
Average number of fœtuses	4·8	Percentage female *cheopis*	40·0
Replenishment rate for 100 rats per day	3·5	Percentage *cheopis*	62·5

TABLE III

Vizagapatam godowns (October-November 1929)

Godowns	Rat density	*X astia* index	*X cheopis* index	Ratio *cheopis* to *astia*	Number of traps with two 'defleaed' rats in each	'Loose' *astia* trapped	'Loose' *cheopis* trapped	Ratio 'loose' *cheopis* to *astia*
Rice and grain	83	2·48	5·13	2·1/1	3	3	25	8·3/1
Groundnuts	88	1·45	0·77	2·5/1	2	1	2	2/1

densities, the general and specific flea indices and the 'loose-flea' indices of these godowns

Though the traps set were few, yet the difference in the results is striking As is seen, the proportion of loose *cheopis* to loose *astia* in the rice godowns is very much higher than the proportion in the groundnut godowns As has been pointed out the grain godowns stock rice and paddy from Berhampore, a highly *cheopis*-infected area, and green gram from the Nizam's Dominions–an endemic centre of plague The groundnuts and myrobalans, on the other hand, come to Vizagapatam from surrounding villages and districts

SUMMARY

(1) Four hundred and forty-three rodents were trapped, out of which 435 were *R rattus*, 7 bandicoots and 1 musk-rat

(2) Out of 2,408 fleas examined, 37 5 per cent were *X astia* and 62 5 per cent were *X cheopis* *X cheopis* is the predominating flea of Vizagapatam

(3) The relative proportion of *cheopis* and *astia* in godowns suggest that *cheopis* is being imported by rail with rice and other grains from the north and west

(4) The increasing trade of Vizagapatam and its developing trade with plague-infected areas like the United and Central Provinces, with its own insanitary conditions as regards rat and *cheopis* infestation all imply danger not only to itself but also to the towns and ports in communication with it

(c) BEZWADA* (November-December 1929)

Bezwada, a municipal town in the Kistna district, lies on the banks of the Kistna River and presents a picturesque appearance with its canals laden with country boats and barges It is an important railway junction for passenger and grain traffic It communicates with Masulipatam on the east coast, with the Nizam's Dominions and Bombay Presidency on its north-west, with Calcutta in the north-east and Madras in the south

Population—The population according to the census of 1921 is 44,159

Housing and sanitation—The town is generally congested except in the extensions where the public offices and Government and other bungalows are situated The houses are mostly contiguous and with a few exceptions are dark and ill-ventilated and afford ample shelter for breeding rats Sanitation is poor

Climate—The climate is generally very hot and dry and in the summer months the temperature rises to somewhere between 115°F–120°F Towards the end of the year, however, it is generally cool and pleasant The average annual rainfall is 37 inches

*This place was surveyed by Dr D S Mankikar

TABLE IV

Rodents and fleas–Bezwada (November-December 1929)

Locality	Number of traps laid	Number of rats trapped	Rat density * (number of rats per 100 traps)	Total number of fleas	General flea index for *R rattus*	*X astia*	*Astia* index for *R rattus*	*X cheopis*
Main bazaar	136	206	152	592	1 74	591	1 74	1
Grain godowns	171	208	122	1,048	2 87	1,027	2 69	21
Rice mills	44	15	34	22	1 30	22	1 30	
Cotton presses	30	8	27	62	7·75	62	7·75	
N G S Rly goods-shed	50	3	6	8	2·66	8	2 66	
M S M Rly goods-shed	30	1	3	2		2		
Railway quarters	24	3	13					
Residential areas	519	271	52	904	2 51	903	2 51	1
TOTAL	1,004	715	89	2,638	2 16	2,615	2 12	23

Percentage female rats	55 8	Percentage female *astia*	56·7
Percentage pregnant females to total females	19·0	Percentage *astia*	99 13
Average number of fœtuses	4 9	Percentage female *cheopis*	47 8
Replenishment rate for 100 rats per day	3 3	Percentage *cheopis*	Less than 1 per cent

* These include 181 *R norvegicus* and 4 bandicoots

Period of survey–9th November to 6th December, 1929
Mean dry bulb temperature 8 A M 78 8° Fahr
" wet bulb temperature 8 A M 71 6° Fahr
" relative humidity 8 A M 69 per cent
" saturation deficiency 8 A M 0 31 inches

History of plague—Bezwada is in close and frequent communication with the Nizam's Dominions where plague is endemic There was one small outbreak of plague in 1917-1918 responsible for about 53 cases Almost every year there have been a few cases imported from the Nizam's Dominions which have been isolated at the station by a special plague inspector and sent to the hospital Otherwise the town has been free from plague since 1918

Imports and exports—Cotton goods from Bombay, dried fruit, hides and skins, cotton, gram, peas and onions from the Nizam's Dominions form the chief imports Paddy, rice and fresh fruit are the chief exports

Rodents—Details are given in Table IV As usual the godowns and bazaar showed a very large rat population A very large number of *R norvegicus*–as many as 181–were trapped All these were from the bazaar, godowns, and houses in the vicinity of godowns Attempts were made to trap as many rats as possible from the goods-sheds of the Nizam's Railway but only 3 rats were trapped These goods-sheds had been regularly fumigated with sulphur dioxide and this may probably account for their low rat density Similarly the railway quarters which are well constructed and which are regularly inspected by the railway sanitary staff harboured few rats

Fleas—Details are given in Table IV It will be seen that the highest general flea index of 7 75 was obtained in the cotton presses All other areas showed indices varying from 1 30 to 2 87 The fleas are practically all *X astia* Only 23 *X cheopis* were seen, out of which 21 were from the godowns and two from the bazaar and houses in the vicinity of the godowns Attempts to trap loose fleas from these godowns were unsuccessful It looks as if *cheopis* were an invading species which so far has failed to establish itself

Table V gives flea indices for the *R norvegicus* caught separately from the *R rattus*

TABLE V

R norvegicus (*caught separately from* R rattus) *Bezwada* (*November-December* 1929)

Locality	*R norvegicus*	Total flea (all *X astia*)	Flea and *astia* index
Main bazaar	58	246	4 24
Grain godowns	30	308	10 26
Residential areas	31	183	5 90
TOTAL	119	737	6 19

SUMMARY

(1) Seven hundred and fifteen rodents were trapped, out of which 530 were *R rattus*, 181 *R norvegicus* and 4 bandicoots

(2) Two thousand six hundred and thirty-eight fleas were examined Two thousand six hundred and fifteen were *X astia* and 23 were *X cheopis* which were nearly all obtained from godowns

3 Bezwada is very exposed to plague infection by rats and fleas from the Nizam's Dominions, so should *X cheopis* ever increase there will be a grave risk of severe plague Probably the one mild epidemic 1917-1918 of only 53 cases was *astia*-borne, as it is unlikely that *cheopis* was more prevalent then than now So far *X cheopis* appears to have been unsuccessful in invading Bezwada

REPORT NO. VIII.

CUDDAPAH AND PRODATTUR IN THE DRY DECCAN AREA NORTH-WEST OF MADRAS

(Tirupathi and Tirumalai whose surveys are reported in Report No VI published in the *Indian Journal of Medical Research* for April 1930 can with advantage be grouped with these)

(A) CUDDAPAH* (September-October 1929)

Cuddapah, a municipal town with a population of 19,600, is the head-quarters of the district of the same name Being on the main railway line running between Madras and Bombay, it is an important trading centre of the district Its altitude is 433 feet

Housing conditions—The majority of the houses are dark and ill-ventilated and have either flat roofs made of rafters and twigs dumped with clay or are roofed with country tiles These and the thatched houses on the outskirts of the town afford good shelter for rats

Climate—The climate is hot and dry The average annual rainfall is 33 inches Meteorological records for the period of the survey are as follows —

Period of survey–5th September to 5th October, 1929

Mean dry bulb temperature at 8 A M 82 52° Fahr
„ wet bulb temperature at 8 A M 74 64° Fahr
„ relative humidity at A M 70 per cent
„ saturation deficiency at 8 A M 0 37 inches

Plague—Plague occurred in 1903 and 1912 but records are not available It again occurred in 1918 and 1919 with mortalities 53 and 41 respectively Thus these epidemics were very small The infection is said to have been imported from Anantapur district on the west There has been no plague since

Exports and imports—The chief exports are cholam, turmeric, groundnut and cotton

*This place was surveyed by Jamadar F Jesudasan, I M D

The chief imports are cereals and pulses from the Bombay Presidency, Nizam's Dominions, Mysore and Northern India

Rodents—Details are given in Table I *R rattus* alone was caught A high rat density of 166 was obtained for the shops and godowns in the market area All the rats were autopsied and the spleen smears examined for *B pestis* with negative results

Fleas—Details are given in Table I One thousand nine hundred and fifty-five fleas were examined All were *astia* The highest flea index observed in the market area was 5 6 Attempts were made to trap loose fleas in the railway godowns by using one dozen 'de-fleaed' rats and as a result three *astia* and a single *cheopis* were caught It is very probable indeed that in the four epidemics of plague reported above the vector was *X astia*

TABLE I

Rodents and fleas–Cuddapah town

Locality	Number of traps laid	Number of rats caught	Rat density (number of rats per 100 traps)	Total *astia*	Other fleas	*Astia* index for *R rattus*
Market area (shops and grain godowns)	84	140	166	843		5 6
Houses in market area	123	78	60	222		2·8
Houses away from the market area	694	263	38	890		3 3
TOTAL	901	481	53	1,955		4 1

Percentage of female rats	52
Percentage of pregnant females to total females	26
Average number of fœtuses	5 4
Replenishment rate for 100 rats per day	6·1
Percentage of female *astia*	51 9

SUMMARY

(1) Four hundred and eighty-one *R rattus* were trapped

(2) *X astia* is the only flea found in the town

(3) Four epidemics of plague have been reported These were probably due to an infection carried by *X astia*

(4) One loose *cheopis* was trapped in a railway godown showing that it tends to be imported

(B) PRODATTUR AND YERRAGUNTLA* (October 1929)

Prodattur, a municipal town in Cuddapah district, is the headquarters of the taluk of the same name It is the chief cotton-producing centre and is situated about eight miles from Yerraguntla, the nearest railway station on the Madras-Bombay line (broad gauge)

Housing conditions—The commonest type of house is one with flat roofs made of rafters and twigs dumped with clay Others have roofs of country tiles or thatch All these afford ample shelter for rats

Climate—The climate is hot and dry The average annual rainfall is 31 inches The meteorological records for the period of survey are as follows —

Period of survey—10th to 17th October, 1929

Mean dry bulb temperature at 8 A M 82 52° Fah
" wet bulb temperature at 8 A M 74 61° Fah
" relative humidity at 8 A M 70 per cent
" saturation deficiency at 8 A M 0 37 inches

Plague—Except for a few imported cases from Anantapur district there has been no plague

Exports and imports—The chief exports are cholam, cambu, ragi, turmeric, groundnuts and cotton The ginned cotton is sent to Madras and Bombay

The chief imports are, rice from Rangoon via Madras, and from Nellore Cereals and pulses from Bombay, Mysore and Northern India

Survey—The localities trapped have been classified into —

(1) The bazaar area, having grain godowns and retail grain shops, (2) Houses other than those in the bazaar area, (3) the four ginning factories, (4) Yerraguntla, a railway station about eight miles from Prodattur, with a few houses around it

Rodents—Details are given in Table II One hundred and twenty-nine *R rattus* were trapped The rat density for the residential area was slightly higher than that for the bazaar area All the rats were autopsied and spleen smears were examined for *B pestis* with negative results

Fleas—Details are given in Table II Six hundred and sixty-one *astia* were examined *X astia* is the only species prevailing in the town The flea index for the residential area was higher than for the bazaar area This may be due to the better construction of the shops and grain godowns in the bazaar It is interesting to note the entire absence of *cheopis* in this town, which collects cotton grown *locally*

SUMMARY

(1) One hundred and twenty-nine *R rattus* were trapped
(2) *X astia* was the only species found *Cheopis* was absent
(3) No plague has occurred

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*This place was surveyed by Jamadar F Jesudasan, I M D

TABLE II

Rodents and fleas–Prodattur and Yerraguntla

Locality	Number of traps laid	Number of rats caught	Rat density (number of rats per 100 traps)	Total fleas	General flea index for *R rattus*	*X astia*	*Astia* index for *R rattus*	*X cheopis*	*Cheopis* index for *R rattus*
Prodattur—									
Bazaar area (with grain godowns)	324	65	21			304	4 4		..
Residential area	234	55	23			318	5 7		
Ginning factories	78	9				39	4 5		
TOTAL	636	129	20			661	5 1		
Yerraguntla railway premises	24	7	29			40			

Percentage of female rats	73	Replenishment rate for 100 rats per day	5 4
Percentage of pregnant females to total females	33	Percentage of female *astia* .	59 9
Average number of fœtuses	4 7		

REPORT NO. IX.

MADANAPALLE, CHITTOOR, VELLORE AND TIRUPATTUR, IN THE REGION WEST OF MADRAS AND NEAR THE BORDERS OF THE MYSORE PLATEAU

(1) Madanapalle Town * (November 1929)

Madanapalle is a town in Chittoor district, having a population of 141,310 It is situated at an altitude of 3 200 feet on the borders of the Mysore plateau

Housing condition—With the exception of a few bungalows the houses are damp, dark and insanitary Most of them have flat roofs of beaten clay and mud floors Others are roofed with country tiles These and the thatched huts located in their midst afford ideal shelter for rats

Climate—The climate is cool and dry The meteorological records for the period of survey are as follows —

Period of survey–20th November, 1929 to 28th November, 1929

Mean dry bulb temperature at 8 A M 71 5° Fahr
" wet bulb temperature at 8 A M 66 4° Fahr
" relative humidity at 8 A M 65 per cent
" saturation deficiency at 8 A M 0 2 inches

Plague—There were outbreaks of plague in 1904 and 1912 when the infection was traced to Kolar in Mysore There has been no plague since

Exports and imports—It has no export of any importance The chief imports are food grains from Mysore and rice from Rangoon via Madras

Rodents—Details are given in Table I Three hundred and thirty *R rattus* were trapped All rats were autopsied and spleen smears examined *B pestis* was not found

Fleas—Details are given in Table I Sixty-seven per cent of the fleas were *X cheopis* Thus *cheopis* is the predominant flea Its index for the residential area (7 1) is much higher than for the bazaar area (4 6) which is unusual but is probably accounted for by the bazaar area having better constructed houses

SUMMARY

(1) Three hundred and twenty *R rattus* were trapped

(2) One thousand three hundred and ninety-five rat fleas were examined, of which 67 per cent were *X cheopis* and 33 per cent were *X astia* *Cheopis* is the predominant flea and is well distributed

(3) Plague has occurred twice–1904 and 1912

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* This place was surveyed by Jamadar F Jesudasan, I M D

TABLE I

Rodents and fleas–Madanapalle

Locality	Number of traps	Number of rats caught	Rat density (number of rats per 100 traps)	Total fleas	General flea index	*X astia*	*Astia* index for *R rattus*	*X cheopis*	*Cheopis* index for *R rattus*	*Pulex irritans*
Bazaar area having grain shops	40	20	50	133	6·6	41	2·5	92	4·6	
Residential areas	240	118	49	1,262	10·7	420	3·5	842	7·1	1
TOTAL	280	138	49	1,395	10	461	3·3	934	6·7	1

Percentage of female rats	73	Replenishment rate per 100 rats per day	11·3
Percentage of pregnant females to total females	50	Percentage of female *astia*	61·8
Average number of fœtuses	5	Percentage of female *cheopis*	42·0

(B) CHITTOOR TOWN* (October-November 1929)

Chittoor, a municipal town with a population of 237,737, is the headquarters of the district of the same name It lies on the metre gauge line of the Madras and Southern Mahratta Railway between the two stations Katpadi and Renigunta on the Madras-Bangalore and Madras-Bombay lines respectively Its altitude is 1,000 feet

Housing conditions—Many of the houses in the central portion of the town have tiled roofs with dung and mud floors There are only a few terraced house with paved floors The houses in the out-skirts of the town are mostly thatched There are some better class bungalows in the eastern portion of the town

Climate—It is hot in summer and pleasantly cool in the cold weather Meteorological records for the period of survey are as follows —

Period of survey–26th October, 1929 to 11th November, 1929
Mean dry bulb temperature at 8 A M 75 5° Fahr
" wet bulb temperature at 8 A M 72 1° Fahr
" relative humidity at 8 A M 73 1 per cent
" saturation deficiency at 8 A M 0 23 inches

Plague—This occurred once in 1912–figures are not available

Exports and imports—The chief exports are jaggery, tamarind and groundnuts The chief imports are rice from Rangoon and Nellore via Madras and cereals and pulses from the neighbouring districts

Survey—As seen from Table II the areas surveyed have been arranged in 5 groups The central bazaar area includes grain godowns

Rodents—Details are given in Table II Three hundred and thirty *R rattus* were trapped The density is high for all areas It is highest in the bazaar area Santhapet comes next with a density of 74 All rats were autopsied and the spleen smears examined for *B pestis* with negative results

Fleas—Details are given in Table II One thousand five hundred and thirty-seven rat-fleas were examined–1,211 *astia* and 326 *cheopis* *X astia* is the prevailing flea and was the only flea found in huts in the central town Santhapet has the highest index, next comes the residential areas

SUMMARY

(1) Three hundred and thirty-nine *R rattus* were trapped

(2) One thousand five hundred and thirty-seven fleas were examined, of which 79 per cent were *X astia* and 21 per cent were *X cheopis*

(3) Plague has occurred only once in 1912

* This place was surveyed by Jamadar F Jesudasan, I M D

TABLE II

Rodents and fleas–Chittoor town

Locality	Number of traps laid	Number of rats caught	Rat density (number of rats per 100 traps)	Total fleas	General flea index for *R rattus*	*X astia*	*Astia* index for *R rattus*	*X cheopis*	*Cheopis* index for *R rattus*
Central town bazaar area	72	100	138·8	558	5·6	433	4·3	125	1·3
Central residential areas	224	103	46	470	1·2	350	3·4	120	1·2
Central huts in residential areas	40	15	37	58	3·9	58	3·9		
Santhapet ½ mile away	40	30	74	140	4·6	88	2·9	52	1·7
3 villages 1 mile away	200	82	41	311	3·8	282	3·2	29	0·3
TOTAL	576	330	57	1,537	4·6	1,211	3·7	326	0·9

Percentage of female rats	70·5	Average number of fœtuses	5·3
Percentage of pregnant females to total females	47·5	Replenishment rate per 100 rats per day	10·7

(c) VELLORE* (April 1930)

Vellore, a municipal town and the headquarters of the North Arcot district, has an area of about four square miles and a population of 50,210 A branch line of the South Indian Railway joins it to Katpadi on the main railway line of the M and S M Railway running from Madras to the south-west It is connected by a good road with Madras and Bangalore

Housing and sanitation—Most of the houses are dark and ill-ventilated and are roofed with country tiles Sanitation, except in the town extensions, is very poor

Climate—The town lies at an altitude of 707 feet above the sea-level The climate is hot and dry, the mean monthly temperature (at 8 A M) in 1929 varied between 70°F in December to 87°F in May The average annual rainfall is 42 inches The meteorological observations during the survey are as follows —

Period of survey–2nd April to 12th April, 1930

Mean dry bulb temperature at 8 A M 86° Fah
" wet bulb temperature at 8 A M 79° Fah
" relative humidity at 8 A M 71 per cent
" saturation deficiency at 8 A M 0 36 inches

Plague—Plague is said to have first appeared in December 1898, to have continued to March 1899 and to have been responsible for about 300 cases, the probable source of infection being Mysore A second outbreak of about 100 cases occurred in 1904-05, and a third outbreak of about 70 cases in 1914-15 Since then, with the exception of a few imported cases, the town has been free At the time of the survey, plague was occurring in Tirupattur in the same district There is passenger and grain traffic between the two places

Exports and imports—Tobacco, maize, boiled rice, tamarind and gingelly oil are the chief exports The chief import is raw rice from Nellore and Guntur districts

Rodents—Details are given in Table III One hundred and fifteen *R rattus* and 1 bandicoot were trapped The bazaar and the godowns showed as usual high rat densities All the rats trapped were autopsied Their spleen smears examined for the presence of *B pestis* were negative

Fleas—Details are given in Table III Both *X astia* and *X cheopis* were present in the town, the former predominating–77 6 per cent The *cheopis* indices for the bazaar and the godowns were 2 8 and 1 8 respectively While *cheopis* were seen in the bazaar areas *none* were found on the rats trapped from houses

SUMMARY

(1) One hundred and fifteen *R rattus* and 1 bandicoot were trapped which (2) Five hundred and forty-two fleas were examined, out of which 22 4 per (3) were *X cheopis* and 77 6 per cent were *X astia* *X astia* is the predoing flea at Vellore *Cheopis* is found only in bazaars and godowns

*This place was surveyed by Dr D S Mankikar

TABLE III

Rodents and fleas–Vellore (April 1930)

Locality	Number of traps laid	Number of rats trapped	Rat density (number of rats per 100 traps)	Total fleas	General flea index for *R rattus*	*X astia*	*Astia* index for *R rattus*	*X cheopis*	*Cheopis* index for *R rattus*
Bazaar	40	29	73	157	5·4	74	2·6	83	2·8
Godowns	40	22	55	142	6·5	104	4·7	38	1·8
Houses	230	65	28	243	2·8	243	2·8		
Railway goods-shed	10								
TOTAL	320	116	36	542	4·2	421	3·1	121	1·1

Percentage of female rats	48·3	Percentage of female *astia*	41·3
Percentage of pregnant rats to female rats	33·9	Percentage of *astia*	77·6
Average number of fœtuses	5·1	Percentage of female *cheopis*	35·5
Replenishment rate for 100 rats per day	5·2	Percentage of *cheopis*	22·4

(D) TIRUPATTUR (NORTH ARCOT DISTRICT) * (April 1930)

Tirupattur is a taluk headquarters and a municipal town in the North Arcot district It has an area of about two square miles and a population of 16,275 It is on the main railway line of the South Indian Railway running from Madras to Mangalore and the Nilgiris

Housing and sanitation—The houses are mostly in a dilapidated condition and are dark and ill-ventilated They are roofed with country tiles and the floors are riddled with rat holes Sanitation is very poor

Climate—Meteorological records are not kept but its climate can be described by saying that it is about 5°F cooler than Vellore as it lies on higher ground Observations for the period of the survey are as follows —

Period of survey—12th April to 21st April, 1930

Mean dry bulb temperature 8 A M 81° Fahr
" wet bulb temperature 8 A M 73° Fahr
" relative humidity 8 A M 69 per cent
" saturation deficiency 8 A M 0 32 inches

Plague—There is a history of plague having occurred in 1902 and 1922 but no records are available Plague started again in December 1929 and continued up to the time of the survey The first rat-fall occurred on 15th December, 1929, in the small bazaar after a consignment of rice had been received from Shimoga in Mysore Plague broke out on 21st December, 1929, and up to 19th April, 1930, 146 attacks and 106 deaths had occurred There were about a hundred rat-falls The town was evacuated and people inoculated Vigorous rat-trapping was also commenced The Mahommedans, who form about 40 per cent of the total population, were the most affected as they would neither submit to inoculation nor evacuate their houses

Exports and imports—Groundnuts, castor seed and tamarind are the chief exports Rice from Nellore and Bezwada and green gram from Trichinopoly are the chief imports Miscellaneous articles come from Madras

Rodents—Details are given in Table IV One hundred and seven *R rattus*, 30 mice and 4 musk-rats were trapped Rat densities are not calculated because the number of rats caught is too small This was because there was opposition to rat trapping from the people All the rats trapped were autopsied Their spleen smears examined for the presence of *B pestis* were found negative

Fleas—Details are given in Table IV Both *X astia* and *X cheopis* were found in all the localities of the town Out of 298 fleas examined 242 or 81 2 per cent were *X cheopis* As far as can be judged *cheopis* is not only the commoner flea but is also very well distributed

SUMMARY

(1) An epidemic of plague is just ending probably because the hot weather is beginning

* This place was surveyed by Dr D, S Mankikar,

TABLE IV

Rodents and fleas–Tirupattur (North Arcot district), April 1930

Locality	Number of traps laid	Number of rodents trapped	Total fleas	General flea index for *R rattus*	*X astia*	*Astia* index for *R rattus*	*X cheopis*	*Cheopis* index for *R rattus*
Bazaar	120	16	17		8		9	
Houses	200	18	61		14		47	
All localities (using municipal mouse traps)	1,600	107	220	2·5	34	0·5	186	2·0
TOTAL	1,920	141	298	2·4	56	0·6	242	1·8

Percentage of female rats	50	Percentage of female *astia*	55·4
Percentage of pregnant female rats	13·9	Percentage of *astia*	18·8
Average number of fœtuses	5·3	Percentage of female *cheopis*	46·3
Replenishment rate for 100 rats per day	2·4	Percentage of *cheopis*	81·2

(2) Two hundred and ninety-eight fleas were examined, out of which 18·8 per cent were *X. astia* and 81·2 per cent were *X. cheopis*. *X. cheopis* is the predominating flea and appears to be distributed throughout the town.

(3) Plague is likely to recur unless vigorous action is taken against rats and in improving sanitation.

REPORT NO. X

RE-SURVEYS OF MADRAS AND NEGAPATAM

(1) Madras City * (December-January 1930)

Survey.—Madras city was surveyed in July-August 1929 and this re-survey was done to note any seasonal change in rats and fleas. The same areas were chosen as last time.

Climate.—The mean monthly temperature at 8 A.M. in 1929 varied from 74°F. in January to 88°F. in May. The meteorological records during the survey were as follows:—

Period of survey—16th December, 1929 to 10th January, 1930.

Mean wet bulb temperature at 8 A.M. 75·3° Fahr.
„ dry bulb temperature at 8 A.M. 72·0° Fahr.
„ relative humidity at 8 A.M. 85·5 per cent.
„ saturation deficiency at 8 A.M. 0·11 inches.

Rodents.—Three hundred and fifty-three rodents were trapped. Three hundred and forty-nine were *R. rattus*, 3 were bandicoots, and 1, a musk-rat. The rat density for the whole city showed an increase from 29 to 35, but the replenishment rate showed a decrease to 4·2 from 5·3.

Rat-fleas.—Two thousand four hundred and forty-eight fleas were collected and of these 2,188 were *X. astia* and 260 *X. cheopis*. The distribution of *X. cheopis* in the city was found to correspond exactly with what had been observed in July-August 1929—limitation to the Harbour and Buckingham Mill areas.

The flea indices in all areas showed a general increase, except Kassimodu which showed a decrease from 6·1 to 3·7. In the two localities where *astia* and *cheopis* both prevail, the latter appear to have undergone a marked numerical increase but not the former. On the other hand in most places where *astia* alone exists, it has increased. The flea indices showed a particularly great increase in the Harbour area, in George Town, and in the cotton mills.

* This place was surveyed by Drs. P. V. George and D. S. Mankikar.

TABLE I

Data of second rat-flea survey of Madras city (December-January 1930)

Places	Number of traps laid	Number of rats caught	Number of rats per 100 traps	Number of fleas obtained	General flea index for *R rattus*	Number of *X astia*	*Astia* index for *R rattus*	Number of *X cheopis*	*Cheopis* index for *R rattus*
Madras harbour									
Rice godowns	67	7	10	71	10·1	5	0·7	66	9·4
Groundnut godowns	45	10	22	67	6·7	67	6·7	0	
Cotton godowns									
B & C Mills	244	32	13	203	6·5	9	0·3	194	6·2
Choolai Mills	60	12	20	135	11·3	135	11·3	0	
George Town rice godowns	90	95	106	936	9·9	936	9·9	0	
Wall tax road paddy godowns	74	52	70	202	3·7	202	3·7	0	
Retail bazaars, Chintadripet and Triplicane	156	86	55	492	5·3	492	5·3	0	
Residential areas									
Perambur	90	30	33	178	5·9	178	5·9	0	
Triplicane	44	7	16	68	9·7	68	9·7	0	
Chintadripet	44	7	16	41	5·9	41	5·9	0	
Kassimodu	91	15	17	55	3·7	55	3·7	0	
TOTAL	1,005	353	35	2,448	6·8	2,188	6·1	260	

Percentage of female rats	66·5	Replenishment rate of 100 rats per day	4·2
Percentage of pregnant rats to total rats	12·7	Percentage of female *astia*	43·1
Average number of fœtuses	5·3	Percentage of female *cheopis*	48·5

(B) NEGAPATAM * (January 1930)

A re-survey of Negapatam town was carried out from 16th January, 1930 to 28th January, 1930 to ascertain the seasonal changes in rats and fleas during these cooler months of the year as compared with the hot months, of June and July, the time of the first survey The mean monthly temperature at 8 A M in 1929 varied from 76°F in December to 87°F in May The average annual rainfall is 55 inches The meteorological records during the survey are as follows —

Mean dry bulb temperature at 8 A M 77° Fahr
" wet bulb temperature at 8 A M 73° Fahr
" relative humidity at 8 A M 76 per cent
" saturation deficiency at 8 A M 0 23 inches

Rodents —(*See* Table II for details) Altogether 226 *R rattus* were trapped Comparing the rat densities with those of the last survey we find, as was to be expected, an increase in the densities in all areas, the difference in the Harbour area coming to 38 The replenishment rate of rats was 6 7 as compared with 3 2 at the last survey

Fleas —(*See* Table II for details) The rat-flea at Negapatam is *X astia* exclusively The Harbour godowns show the highest index of 7 24, an increase of 2 84 fleas per rat over the last survey The residential areas show only a slight increase

TABLE II

Rodents and fleas–Negapatam (January 1930)

Locality	Number of traps laid	Number of rodents trapped	Rat density, i e, number of rats per 100 traps	Total fleas *X astia*	General and specific *astia* index
Port godowns	171	141	82	1,021	7 24
Railway goods-shed	22	1		8	
Negapatam bazaar	44	25	57	82	3 28
Nagore bazaar	44	17	39	53	3·11
Negatapam houses	110	19	17	71	3 73
Nagore houses	44	23	52	81	3 52
Barges	44				
TOTAL	479	226	47	1,316	5 82

Percentage of female rats	57·5
Percentage of pregnant rats to total females	30 0
Average number of fœtuses	6 2
Replenishment rate for 100 rats per day	6 7
Percentage of female *astia*	48

* This place was surveyed by Dr D S Mankikar

CONCLUSION

A re-survey in the cold weather confirms the finding of a survey in the hot weather that *X astia* is the sole rat-flea of Negapatam This is interesting, because, as stated in the first report, there was an epidemic of 154 cases in 1913-14 probably following infection from a ship from Rangoon, which ship later also infected Colombo So, unless *cheopis* was prevalent then and has since died out, which is unlikely, this epidemic of 1913-14 was *astia*-borne

REPORT NO XI

TINNEVELLY, TUTICORIN AND MADURA ON THE SOUTH COASTAL PLAIN

(A) TINNEVELLY AND PALAMCOTTAH * (September-October 1929)

Tinnevelly is a municipal town having a population of 53,783 It consists of three divisions, the Tinnevelly town proper and two suburbs, Pettai and Veeraraghavapuram, which are situated on either side of the main town and are cut off from it by long stretches of paddy fields Veeraraghavapuram is the railway station called Tinnevelly junction

Palamcottah is another municipal town, and is the headquarters of the Tinnevelly district It has a population of 46,643 It is only two miles distant from Tinnevelly, and is separated from it by the river Tamparavarni

Climate—It has a light rainfall and a high equable temperature–the average mean temperature is the highest for any district in the presidency The average annual rainfall is about 28 inches, of which over 18 inches are received during the north-east monsoon from October to December The meteorological observations made during the period of survey are as follows —

Period of survey–28th September to 24th October, 1929

Mean dry bulb temperature 9 A M 82° Fahr
„ wet bulb temperature 9 A M 75 6° Fahr
„ relative humidity 9 A M 74 per cent
„ saturation deficiency 9 A M 0 3 inches

Housing and sanitation—All types of houses are to be seen Terraced houses are common The residential areas in Tinnevelly are much overcrowded, but in Palamcottah most of the houses have extensive grounds around The general sanitation in Palamcottah is good, on the other hand, owing to busy trade and congestion in residential quarters, the sanitation of Tinnevelly is very poor

Commerce—Pettai appears to be the busiest commercial centre, and the *District Gazetteer* describes its trade thus —'Almost all articles (*except cotton and jaggery*) exported from the west and centre of the district, most articles imported from outside the district between north and south or east and west, go through the hands of the Pettai merchants Onions, chillies, bones, gingelly,

* These two places were surveyed by Dr P V George

TABLE I

Rodents and fleas–Tinnevelly and Palamcottah (September-October 1929)

Places	Number of traps laid	Number of rats caught	Rat density (number of rats per 100 traps)	Total fleas obtained	Total flea index for *R rattus*	Number of *X astia*	*Astia* index for *R rattus*	Number of *X cheopis*	*Cheopis* index for *R rattus*
Pettai Bazaar	232	136	59	201	1·48	201	1·48	0	
Pettai houses	40	8		20		20		0	
Tinnevelly town bazaar	194	271	140	1,252	4·62	956	3·52	296	1·10
Tinnevelly town houses	328	133	41	251	1·88	245	1·81	6	
Veeraraghavapuram Bazaar	65	22	34	101	4·59	67	3·01	34	1·55
Veeraraghavapuram houses	43	21	49	111	5·28	110	5·21	1	
Palamcottah Bazaar	53	48	91	232	4·83	232	1·83	0	
TOTAL	955	639	67	2,168	3·39	1,831	2·86	337	0·53

Percentage of female rats	57	Replenishment rate of 100 rats per day	4·9
Percentage of pregnant rats to total rats	14·7	Percentage of female *astia*	51
Average number of fœtuses	5·3	Percentage of female *cheopis*	31

grains of all kinds, pour in from all parts of the district for export either to another part of the district or beyond it'

The grain trade, however, is not considerable and the population largely subsists on the district produce Sahyar Street in Tinnevelly town is the centre of the grain godowns It is to be noted that the chief difference between the trade of Pettai and Tinnevelly town proper, lies in the fact that all trade in cotton and cotton goods is centralized in the latter Cotton is imported into the place largely from Koilpatty, Papanasam, Madura and Coimbatore, while cotton seed is received also from Bellary, Hubli, Dharwar, etc

Plague—These two towns have not been visited by plague so far, and except for a single epidemic in Tuticorin in 1923-24, the whole district has been singularly free

Rodents—Six hundred and thirty-nine rodents were trapped, and of these 634 were *R rattus*, 3 musk-rats, and 2 bandicoots Table I gives the rat density, etc, for the different areas It will be seen that the rat density was found to be high in all areas, and much higher in Tinnevelly town bazaar, viz, 140–there has been no systematic rat destruction practised here

Among the rats examined 57 per cent were females, and the replenishment rate for 100 rats per day was found to be 4 9

Rat-fleas—Two thousand one hundred and sixty-eight fleas were examined and of these 1,831 were *X astia*, and the remaining 337 were *X cheopis* Among *astia* 51 per cent were females, but among *cheopis* only 31 per cent were females

Table I gives the flea indices for the different areas Generally the flea indices were comparatively low, considering the season of survey The highest index of 5 28 was obtained in the municipal sweeper lines at Veeraraghavapuram

The distribution of *cheopis* was found practically *limited to the bazaar streets* of Tinnevelly town and Veeraraghavapuram Out of the small total of 7 *cheopis* obtained from residential areas, 6 were from weaver houses The total absence of *cheopis* in Pettai and Palamcottah Bazaars and from almost all residential areas is to be noted Further, even in the Tinnevelly town bazaar, *cheopis* were absent from Sahyar Street, the centre of the wholesale grain trade As has been observed, the cotton trade is limited to Tinnevelly town These facts strongly suggest that *cheopis* is an imported flea and that such importation has taken place through cotton The presence of *cheopis* in Veeraraghavapuram Bazaar may be explained by its nearness to the railway goods-shed which receives all the cotton imported to Tinnevelly

SUMMARY

(1) Six hundred and thirty-nine rodents were examined, and of these 3 were musk-rats, 2 bandicoots and the rest, *R rattus*

The rat density was uniformly high, and very high (140) in Tinnevelly Bazaar

(2) Two thousand one hundred and sixty-eight fleas were collected and identified Of these 1,831 were *X astia* and 337 were *X cheopis* The

distribution of *cheopis* was found practically limited to the bazaar streets of Tinnevelly town and Veeraraghavapuram *X cheopis* seems to be an imported flea, and the evidences suggest that it has been imported with cotton from adjoining districts and the Bombay Presidency

(3) There has been no human plague

(B) TUTICORIN * (October-November 1929)

Tuticorin, a municipal town in Tinnevelly district, is a terminus of the South Indian Railway, and is the chief seaport for the export trade of the southern districts of the presidency The harbour is formed by a low sandy cape and lies within a circular chain of islands and reefs Fairly large coasting craft can enter within the reefs, but bigger vessels have to anchor in the roadstead six or seven miles from the town The town has a population of 44,522 in an area of 1 8 square miles and is thus one of the most densely populated towns in the presidency

Climate—The climate is hot and comparatively dry The average annual rainfall is only 22 11 inches, and of this about 16 inches is received during the north-east monsoon The meteorological records during the period of survey are as follows —

Period of survey–26th October to 30th November, 1929

Mean dry bulb temperature 9 A M 80 3° Fahr
„ wet bulb temperature 9 A M 75 2° Fahr
„ relative humidity 9 A M 79 per cent
„ saturation deficiency 9 A M 0 24 inches

Housing and sanitation—The residential areas to the north of the railway station are much overcrowded The houses afford ample shelter for rats, both in the floors and in the ceiling There are no rat-proof godowns in the town The sanitation is very unsatisfactory in all parts

Commerce—Exports are much heavier than imports–cotton and yarn are the chief exports and next come tea, coffee, chillies and onions The chief imports are rice from Rangoon, wheat, barley, pulses, etc, from Bombay, Karachi and Calcutta

Tuticorin receives cotton mainly from its own district of Tinnevelly, and also from Madura, Coimbatore and Ramnad There are several cotton ginning and pressing mills in the town

Plague—Tuticorin has had only one epidemic of plague in 1923-24 with 411 attacks and 230 deaths This epidemic was traced to Madura It caused such a panic that the town was practically emptied

Survey—The survey was done in October-November 1929 during the rainy season All representative areas were surveyed and also the small village of Karappadu in the outskirts of the town

Rodents—Five hundred and thirty-two rodents were trapped, of which 511 were *R rattus*, 5 bandicoots, 5 musk-rats, 2 house mice and 9 gerbilles

* This place was surveyed by Dr P V George

Most of the gerbilles were dug out of rat holes to secure specimens, and we are indebted to the Bombay Natural History Society for its identification as *Gerbilles tatera cuveri* Musk-rats and bandicoots abound

Table II gives the rat density, etc, for the different types of areas in the town The density was highest (69) in the grain godowns in South Raja Street and Beach Road Cotton godowns showed only a small rat density, evidently because they were well constructed and maintained Female rats predominated in all areas, and among them 26 per cent were pregnant The replenishment rate for 100 rats per day was calculated according to the formula published in *U S A Public Health Reports*, Vol XL, No 9, of March 1929 This was found to be 5 3 All the rats were autopsied, and their spleen smears examined All were negative for plague

Fleas—Of a total of 3,016 rat-fleas 1,842 were *X astia*, 1,172 *X cheopis* and 2 *Ctenocephalus felis*

Table I gives the flea indices, etc, for the different representative areas in the town

It is to be noted that very high flea indices (14 5) were obtained in some of the cotton godowns The lowest flea index (1 3) was also got from a few cotton godowns of Messrs A & F Harvey, situated near the Beach These godowns, unlike the others in the town, are built with concrete floors, and they are well lighted and ventilated This difference in construction probably explains the difference in flea indices

In all areas as regards both *astia* and *cheopis* males predominated

The distribution of *cheopis* was found to be strictly localized to the areas of the wholesale trade in cotton and grains *Astia* was the sole flea obtained from gerbilles (which are field rats), further it was the sole flea obtained from residential areas from Karappadu village, and even from the retail bazaar areas This definite difference in distribution of the two species strongly suggests that *cheopis* is imported into this town and that such importation is through cotton and grains Now, the town is a small one extending only 1 8 square miles, and the cotton godowns are not crowded together but are irregularly distributed in and about the town, so the absence of *cheopis* from the residential areas is remarkable This suggests either a very strict limitation of the movement of rats, or that the conditions are unfavourable for the spread of *cheopis*, in which case probably the *cheopis* infestation of the mills is largely maintained by importation

Since it is unlikely that the rat-flea population of the residential areas in 1923-24 differed materially from the present in being wholly *X astia*, it is probable that the small epidemic of that year which affected less than 1 per cent of the population and which was not restricted to any one quarter of the town was *astia*-borne as regards its maintenance The fact that the epidemic did not recur lends additional support to this deduction The importation may or may not have been due to *cheopis*

TABLE II

Rats and fleas–Tuticorin (October-November 1929)

Places	Number of traps laid	Number of rats caught	Number of rats for 100 traps or rat density	Number of fleas gathered	General flea index for *R rattus*	Number of *X astia*	*Astia* index for *R rattus*	Number of *X cheopis*	*Cheopis* index for *R rattus*	REMARKS
Grain godowns	391	270	69	1,431	5·4	831	3·2	599	2·3	South Raja Street and Beach Road
Cotton godowns	364	62	17	520	8·4	133	2·1	387	6·2	Port Press Volkart Bros., Julgam Co., etc
Bazaar area	205	81	40	369	4·6	359	4·4	10	0·12	Great Cotton Road, Big Bazaar Street, etc
Dhal mills	154	18	12	32	1·8	32	1·8	0		Devanpatnam
Ry goods-shed	48	3	6	5		5		0		
Barges	49	4	8	0		0		0		
Residential areas	318	88	28	395	4·5	393	4·4	1		Emperor Street, George Road, Karappadu, etc
TOTAL	1,529	526	34	2,752	5·2	1,753	3·3	997	1·9	

Percentage of female rats	61	Replenishment rate for 100 rats per day	5·3
Percentage of pregnant female rats	26	Percentage of female *astia*	17·9
Percentage of pregnant to total rats	15·3	Percentage of female *cheopis*	35·4
Average number of fœtuses	5·5		

Loose flea experiments—Rats were anæsthetized with petrol and then thoroughly combed and searched for fleas The thoroughness of de-fleaing rats by this method was verified several times It was found that some rats died later on, and so instead of having only one rat in each trap, two or three rats were put into the same trap Immediately after de-fleaing the rats, the traps into which they were put were suspended far above ground in an almost rat-proof room in the laboratory These traps were well secured against ingress of new rats and were left for a night in various godowns The number of traps used was in proportion to the area of the godown Empty baited traps were also put in the same godown at the same time

Similar experiments were performed in a few residential houses as a control

In the morning the traps were collected and put into canvas bags and brought to the laboratory Fleas were collected using the same technique as was employed in our survey No fleas were found on rats left in residences while the rats caught in the traps from the same houses yielded plenty of fleas But in the godowns many loose fleas were caught as seen from Table III

The loose flea index has been calculated per trap and not per rat This has been done because for comparative purposes we wish to get an index that will serve to measure the number of loose fleas per given area which area is that surrounding a trap within the limits that allow a flea to reach a trap The flea index per rat in this case would not serve since the number of surviving rats in the traps was not constant

The results in Table III show that we get great differences in loose flea densities in different areas The loose cotton godowns were easily first, next pressed cotton and rice godowns, and last dhal godowns and residences As was to be expected, this order is practically the same as the order of the attached flea densities of different areas in Table I It is probable that differences between localities in this respect are to be ascribed to the differences in facilities for the shelter and breeding of fleas while off rats Thus cotton and cotton dust seem to be by far the best sheltering material particularly in the humid atmosphere of cotton mills

Summary

(1) Five hundred and thirty-two rodents were trapped during the survey, out of which 511 were *R rattus*, 5 bandicoots, 5 musk-rats, 2 house mice and 9 gerbilles The rat density was highest in the grain godowns

(2) Three thousand and sixteen rat-fleas were gathered, of which 1,842 were *X astia*, 1,172 *X cheopis*, and 2 *Ctenocephalus felis* The flea density was the highest in the cotton godowns Among both *astia* and *cheopis* collected off rats males predominated, whereas among the loose *astia* and *cheopis* caught from godowns females predominated

(3) The loose flea results show the importance of cotton in harbouring fleas.

TABLE III

Loose flea experiments–Tuticorin

Places	Number of traps with 'de-fleaed' rats	Number of loose fleas caught	General loose flea index per trap	General attached flea index	*X astia*			*X cheopis*		
					Number of loose *astia*	Loose *astia* index per trap	Attached *astia* index	Number of loose *cheopis*	Loose *cheopis* index per trap	Attached *cheopis* index
Loose cotton godowns	12	160	13·33	12·42	32	2·66	2·05	128	10·66	10·37
Pressed cotton godowns	20	24	1·20	5·6	13	0·65	3·33	11	0·55	2·22
Grain godowns	29	78	2·7	6·3	43	1·48	3·75	35	1·21	2·50
Dhal godowns	11	2	0·18	1·8	1		1·77	1		
Houses	6	0		7·9	0			0		

(4) There has been only one epidemic of plague in the town, in 1923-24, which caused 230 deaths This epidemic was most probably *astia*-borne, since at present *cheopis* is absent from residential areas

(5) The *cheopis*-infected areas are a source of danger to the rest of the town, because it is probable that *cheopis* is still being imported into them and so infection may also be imported Rat proofing of godowns is urgently indicated

(c) MADURA TOWN * (January-February 1930)

Madura, a municipal town, is the headquarters of the Madura district with a population of 138,894 It is the second largest town in the Madras Presidency and is situated on the right bank of the river Vaigai Being on the main line of the South Indian Railway, it is an important trading and pilgrim centre Recently it has been connected by a narrow gauge line to the Kambam valley. a grain-producing centre and an endemic plague focus

Housing and sanitation—All types of buildings are to be seen but the majority are terraced and have paved floors The eastern and northern portion of the town contain detached bungalows and houses The poorer classes usually live in huts on the outskirts of the town There are no rat-proof godowns

Climate and rainfall—The climate is hot and dry The mean monthly 8 A M temperature varies from 88 8°F in May to 78°F in January The average annual rainfall is 33 8 inches The meteorological records during the survey are as follows —

Period of survey–28th January to 28th February, 1930
Mean dry bulb temperature at 8 A M 77 0° Fahr
„ wet bulb temperature at 8 A M 70 7° Fahr
„ relative humidity at 8 A M 68 per cent
„ saturation deficiency at 8 A M 0 3 inches

Plague—The first outbreak of plague in an epidemic form was in 1911, infection being traced to Palni and Coimbatore From 1920 to 1925 plague occurred every year yielding the following number of deaths in the respective years, 128, 571, 33, 122, 6, 36

Exports and imports—The chief crops raised in the district are ragi, kambu, cholam, gingelly seeds, groundnut and cotton Most of the grain is brought into the town from the Kambam valley. This influx of grains takes place during January, February and March Grains from Bombay, Rangoon and Northern India are also exported via Tuticorin and rice from Nellore and Rangoon Ginned and baled cotton comes from Dindigul, Ramnad, Tinnevelly and Coimbatore

* This place was surveyed by Jamadar F Jesudasan, I M D

Rodents—Details are given in Table IV One thousand eight hundred and nine *R rattus* were trapped It will be seen that all areas showed high rat densities The highest–168–being obtained in the Rajah and 102 in the Madura mill The grain godowns come next with a density of 72 All the rats autopsied and their spleen smears examined for *B pestis* with negative results

Fleas—Details are given in Table IV Both *X astia* and *X cheopis* were present While *astia* prevailed everywhere, the distribution of *cheopis* was confined to certain cotton mills and grain godowns and houses near them The Madura mill, the highest and oldest in the town, showed a *cheopis* index of 9 8, which is the highest obtained It is interesting to contrast this with the finding in the other mills The Rajah mill which after a long stoppage of work reopened in June 1929 had no *cheopis* The Meenakshi mill, which has been reconstructed and started work about a year ago, had one *cheopis* flea The Pandian mills, which after a long stoppage started work in June 1929, had four *cheopis* It is to be noted that this mill is under the same management as the Madura mill and gets its cotton from that mill

The houses in the area around the Rajah mills had 29 *cheopis* out of a total of 307 fleas, but this is probably due to them bordering on the grain godown area Houses around the other mills showed a very small number of *cheopis* The grain godowns showed a *cheopis* index of 3 3 As stated, *cheopis* was also present in houses around these godowns, a few in houses near the grain bazaar and none at all in houses further away

From Table I it is seen that three-fourths of the total *cheopis* was obtained in the Madura mills and in grain godowns The remaining *cheopis* are so few as to be almost negligible compared with the large number of *astia* Thus *astia* is the predominant flea *Cheopis* appears to be an invader associated with cotton and grains This is clearly shown by the facts already mentioned and perhaps made more clear by the following summary —

	Cheopis index
Madura mills (cotton mills)	9 8
Grain godowns	3 3
Houses near grain godowns	1 1
In bazaar	0 4
Near [illegible]	0 1
And from	Nil

TABLE IV

Rats and fleas–Madura town (January-February 1930)

Locality	Number of traps laid	Number of rats caught	Rat density (number of rats per 100 traps)	Total fleas	General flea index for *R rattus*	*X astia*	*Astia* index for *R rattus*	*X cheopis*	*Cheopis* index for *R rattus*
Madura mills	48	49	102	532	10·9	52	1·1	480	9·8
Houses around Madura mills	375	132	35	487	3·7	484	3·7	3	
Rajah mills	24	26	168	153	5·9	153	5·9	Nil	
Houses around Rajah mills	174	98	56	338	3·4	309	3·1	29	0·3
Meenakshi and Pandian mills	96	35	36	171	4·9	166	4·7	5	
Houses around Pandian mills	567	225	39	987	4·3	984	4·3	3	
Grain godowns	160	115	72	713	6·2	329	2·9	384	3·3
Houses around grain godowns	225	59	26	304	5·1	237	4·7	67	1·1
Retail grain bazaar	565	310	55	984	3·2	858	2·8	126	0·4
Houses around retail grain bazaar	1,438	663	46	2,744	4·1	2,668	0·4	76	0·1
Houses away from grain godowns and bazaar	240	97	40	535	5·5	535	5·5	Nil	
TOTAL	3,912	1,809	62	7,948	4·3	6,775	3·7	1,173	0·6

Percentage of female rats	66·5	Replenishment rate per 100 rats per day	3·8
Percentage of pregnant females	17·2	Percentage of *astia* females	46·5
Percentage of pregnant females to total rats	11	Percentage of *cheopis* females	31·2
Average number of fœtuses	5·6		

The loose flea work shown in Table V confirms the great prevalence of *cheopis* in grain godowns and in the Madura mills The absence of fleas in yarn godowns may be partly associated with yarn being stored in better godowns

TABLE V

Loose flea experiment—Madura

Locality	Rat density	*X cheopis* index	*X astia* index	Number of 'de-fleaed' rats used	Loose *cheopis* trapped	Loose *astia* trapped
Madura mills, cotton godowns	102	9·8	1 1	21	16	1
Madura mills, yarn godowns				12	Nil	Nil
Town yarn godowns				12	Nil	Nil
Town grain godowns	42	2·4	0·1	6	25	5

SUMMARY

(1) One thousand eight hundred and nine *R rattus* were trapped

(2) Seven thousand nine hundred and forty-eight fleas were examined, 85 2 per cent were *X astia* and 14 8 per cent were *X cheopis*

Cheopis is practically confined to the Madura mill and to grain godowns and houses nearby

REPORT NO. XII

SALEM, YERCAUD, TRICHINOPOLY AND COIMBATORE IN THE CENTRAL SOUTHERN AREA OF THE PRESIDENCY

(A) SALEM TOWN* (February-March, 1930)

Salem, a municipal town with a population of 52,244, is the headquarters the Salem district It is an important trade centre, being situated at the action of the Bangalore, Trichinopoly and Cuddalore Roads, and being on the ad gauge line of the South Indian Railway from Madras to the west coast

Housing conditions—Except in the town extensions, the houses everywhere dark and ill-ventilated and are roofed with country tiles On the outskirts e town we find only mud huts with thatched roofs which afford ideal ions for the breeding of rats

* This place was surveyed by Jamadar F Jesudasan, I M.D.

Climate and rainfall—The average monthly mean temperature varies from 88°F in April to 75°F in December The average annual rainfall is 39 inches The meteorological records during the survey are as follows —

Period of survey–27th February to 26th March, 1930

Mean dry bulb temperature at 8 A M 78 5° Fahr
" wet bulb temperature at 8 A M 72 4° Fahr
" relative humidity at 8 A M 69 per cent
" saturation deficiency at 8 A M 0 31 inches

Plague—From 1902 to 1905 there were only a few cases of plague imported from Mysore, but from 1909 to 1921 there were several severe outbreaks responsible for 6,000 deaths in all

Exports and imports—The chief exports are groundnuts and ginned cotton The chief imports are cereals and pulses from Mysore, Bombay Presidency and Bengal, rice from Rangoon and Cocanada

Survey—The areas have been classified to show the relation of flea prevalence to the grain and cotton trades

Rodents—Details are given in Table I Four hundred and thirty-six *R rattus* were trapped The grain godowns showed the highest rat density–50 in the town Huts on the outskirts came next with a rat density of 47 All other areas showed rat densities varying between 8 and 33 A rat-catching campaign has been carried on for 10 years despite which, as seen, the rat densities are practically normal There are no rat-proof godowns

Fleas—Details are given in Table I Both *X astia* and *X cheopis* were found in the town *Cheopis* is the predominant flea in the town proper and has a wide distribution, but *astia* is the predominant flea in the huts around the town pointing to its being the indigenous flea There was no great difference between the *cheopis* indices of grain godowns and the surrounding areas (3 and 3 2) A *cheopis* index of 2 6 was obtained in two villages outside the municipal limits All these facts show that *cheopis* distribution in Salem is no longer dependant on grain movements but that *cheopis* has become successfully established in the town On the other hand the association of *cheopis* with grain is shown by the results of loose flea experiments which are as follows —

Locality	Number of 'de-fleaed' rats	Loose *cheopis*	Loose *astia*	Ratio between *cheopis* and *astia* indices in the same localities
Cotton godowns	16	3	3	1/0·8
Grain godowns	9	20	1	3/0·8

As seen, the proportion of loose *cheopis* to *astia* caught in grain godowns was five times higher than the proportion of *cheopis* to *astia* caught on rats

TABLE I

Rodents and fleas–Salem town (March 1930)

Locality	Number of traps laid	Number of rats caught	Rat density (number of rats per 100 traps)	Total fleas	General flea index for *R rattus*	*X astia*	*Astia* index for *R rattus*	*X cheopis*	*Cheopis* index for *R rattus*
Grain godowns	187	95	50	354	3·8	72	0·8	282	3
Houses around grain godowns	220	54	24	258	4 8	84	1 5	171	3·2
Retail grain shops	184	17	9	52	3	12	0 7	10	2·3
Houses around retail grain shops	256	53	21	114	2 1	41	0·8	70	1 3
Cotton ginning factories,	59	5	8	9		4		5	
Houses near cotton gins	246	36	10	164	4 5	108	3	56	1 5
Houses away from grain areas	266	87	33	345	3·9	116	1 3	229	2·6
Huts away from grain areas	60	60	47	272	4 5	213	1	29	0·5
Two villages outside municipal limits	126	30	24	129	4·3	55	1·8	71	2·5
TOTAL	1,604	437	27	1,697	3·7	738	1 7	959	2·0

Percentage of female rats	64	Replenishment rate per 100 rats per day	7
Percentage of pregnant females	92	Percentage of *astia* females	62·1
Percentage of pregnant females to total rats	23	Percentage of *cheopis* females	43 1
Average number of fœtuses	5		

This might have been due to superior activity on the part of *cheopis*, but it is doubtful whether it was wholly so

SUMMARY

(1) Four hundred and thirty-seven *R rattus* were trapped

(2) One thousand six hundred and ninety-seven fleas were examined, of which 57 per cent was *X cheopis* and 43 per cent *X astia* *Cheopis* is the predominating flea in the town proper where it is well distributed

(3) Several severe outbreaks of plague have occurred in the past So far as we can see, there is no reason why they should not recur in the future The present rat campaign does not seem to be very successful

(B) YERCAUD TOWN * (March-April 1930)

Yercaud, a hill station on the Shevaroys in the Salem district, lies at an altitude of 4,500 feet and is connected with Salem by a good road

Housing and sanitation—Except for the bungalows of Europeans the houses are mostly dark and ill-ventilated and are roofed with country tiles and have dung and mud floors Sanitation is bad in the poor class areas

Climate and rainfall—The midday temperature varies from 70°F in May (hot weather) to 67°F in December The annual average rainfall varies from 60 to 80 inches The meteorological observations during the survey are as follows —

Period of survey–27th March to 8th April, 1930

Mean dry bulb temperature at 8 A M 67 9° Fahr
„ wet bulb temperature at 8 A M 64 1° Fahr
„ relative humidity at 8 A M 93 per cent
„ saturation deficiency at 8 A M 0 04 inches

Plague—There were outbreaks of plague in 1910 and 1919–no records of cases are available

Exports and imports—The chief produce is coffee which is exported in large quantities Grains and other articles are usually brought from Salem

Survey—The findings have been tabulated for (1) poor class Indian residences, and (2) bungalows Five hamlets outside Yercaud limits were also trapped but unfortunately no rats were caught

Rodents—Details are given in Table II Only 37 *R rattus* were trapped No explanation can be given for this poor number of rats obtained except that the trapping with wonder traps was very unsuccessful for some unknown reason Rats seemed to be abundant

Fleas—Details are given in Table II The rat fleas prevailing at Yercaud are *X brasiliensis* and *X astia*, the former predominating The houses of the poor classes showed a higher flea index than the bungalows Practically all the fleas collected from bungalows were *X brasiliensis* Twenty *Pulex irritans* were

* This place was surveyed by Jamadar F Jesudasan, I M D

TABLE II

Rodents and fleas–Yercaud (April 1930)

Locality	Number of traps laid	Number of rats	Rat density (number of (rats per 100 traps)	Total fleas	General flea index	*X astia*	*Astia* index	*X brasiliensis*	*Brasiliensis* index
Poor class Indian residences	180	23	12	212	9·2	52	2·3	160	6·9
European and other bungalows	100	14	14	44	3·1	1		43	3·1
TOTAL	280	37	13	256	6·9	53	1·4	203	5·5

Percentage of female rats	70·3	Replenishment rate for 100 rats per day	3·7
Percentage of pregnant females	11·5	Percentage of *astia* females	67·5
Percentage of pregnant females to total rats	8·1	Percentage of *brasiliensis* females	41·3
Average number of fœtuses	7·3	Percentage of *Pulex irritans* females	60

collected from the mud floors of houses by picking them off the floors They appear to be extremely numerous

SUMMARY

(1) Only 37 *R rattus* were trapped

(2) Total rat-fleas collected 256–*brasiliensis* 70 per cent, *astia*, 21 per cent *Pulex irritans* was very common

(3) There were two outbreaks of plague in 1910 and 1919 probably carried on by *X brasiliensis*

(c) TRICHINOPOLY * (December 1929 to January 1930)

Trichinopoly, a municipal town with a population of 120,422, is the head-quarters of the district of the same name It is an important junction on the main railway line of the South Indian Railway from Madras to the south It is connected by a branch line with Erode on the Madras-Mangalore and Nilgiris line

Housing conditions—Most of the houses are roofed with country tiles and are dark and ill-ventilated and afford ample shelter for the breeding of rats

Climate and rainfall—The climate is generally hot and dry The average annual rainfall is 33 inches The meteorological records during the survey are as follows —

Period of survey–27th December, 1929 to 26th January, 1930

Mean dry bulb temperature at 8 A M 75 4° Fahr
" wet bulb temperature at 8 A M 70 5° Fahr
" relative humidity at 8 A M 75 per cent
" saturation deficiency at 8 A M 0 22 inches

Plague—Except for a few imported cases from Coimbatore, there is no history of any epidemic

Imports and exports—Paddy, groundnuts and castor oil seeds are largely exported to various places Cotton is exported to Tuticorin, Coimbatore and Ramnad and oilcakes to Colombo The chief imports are cereals and pulses from Mysore, Bombay Presidency, and Northern India and rice from Rangoon and Cocanada

Survey—The town practically consists of only two areas, the central market area with bazaar and godowns and the outer residential area

Rodents—Details are given in Table III Four hundred and forty-nine *R rattus* were trapped The highest rat density of 70 was obtained in the grain godowns All the rats trapped were autopsied and their spleen smears examined *B pestis* was not found

Fleas—Details are given in Table III In all 2,895 fleas were examined –2,843 *X astia* and 52 *X cheopis* Thus the predominant flea at Trichinopoly is *X astia* *Cheopis* was found *exclusively* in the central grain godowns area

* This place was surveyed by Jamadar F Jesudasan, I M D

TABLE I

Rats and fleas–Trichinopoly (January 1930)

Locality	Number of traps laid	Number of rats caught	Rat density (number of rats per 100 traps)	Total fleas	General flea index	*X astia*	*Astia* index for *R rattus*	*X cheopis*	*Cheopis* index for *R rattus*
Central market area (grain godowns)	96	67	70	342	5 1	290	4 3	52	0 8
Residential area	1,004	387	38	2,553	6 2	2 553	6 2		
TOTAL	1,100	454	41	2,895	6 1	2,813	6	52	0 1

Percentage of female rats	53 5	Replenishment rate for 100 rats per day	5 8
Percentage of pregnant females	49 1	Percentage of *cheopis* females to total *cheopis*	32 7
Percentage of pregnant females to total rats	34 5	Percentage of *astia* females to total *astia*	65 9
Average number of fœtuses	2·7		

showing very clearly that it is a foreign invader Some loose flea work was done with results as follows —

Locality	Number of 'de-fleaed' rats	Loose *cheopis*	Loose *astia*	Loose *brasiliensis*	Ratio between *cheopis* and *astia* indices in the same localities
Grain godown, market area	12	15	11		0·2/1
Railway godown for grain and merchandise	12	3	27	1	0/1

As seen the proportion of loose *cheopis* to loose *astia* in the grain godowns was six times the proportion of *cheopis* to *astia* found on rats It is interesting to note that one specimen of *X brasiliensis* was found in the railway godowns, showing how this flea is being imported

SUMMARY

(1) Four hundred and forty-nine *R rattus* were trapped None showed signs of plague

(2) Two thousand eight hundred and ninety-five fleas were examined of which 99 per cent were *astia* and 1 per cent *cheopis* *X astia* with an index of 6 is the prevailing flea A few *cheopis* were found in the central market area only and the one loose *brasiliensis* was caught in a railway godown These two species have been imported *Cheopis* now seems to be moderately well established in the central area

(3) There has been no plague so far

(D) COIMBATORE* (January-February 1930)

Coimbatore, a municipal town, is the headquarters of the district of the same name It has an area of about eight square miles and is an important industrial centre with its cotton mills, ginning presses and large tea and coffee clearing houses It has railway communications with the Nilgiris, the west coast, Madras and the south

Population—The population according to the census of 1921 is about 66,000

Housing and sanitation—Except in the town extensions, the houses generally are dark and ill-ventilated and adjoin one another They are mostly roofed with country tiles and afford facilities for the breeding of rats The place is fairly clean and sanitary

Climate and rainfall—Coimbatore has a healthy climate and is comparatively cool throughout the year The mean monthly temperature (8 A M) in

*This place was surveyed by Dr D S Mankikar

1929 varied from 72°F in January to 82°F in May The average annual rainfall is about 25 inches

Period of survey–29th January, 1930 to 26th February, 1930

Mean dry bulb temperature at 8 A M 73° Fahr
" wet bulb temperature at 8 A M 70° Fahr
" relative humidity at 8 A M 82 per cent
" saturation deficiency at 8 A M 0 15 inches

Exports and imports—The chief produce of the district is cotton, cholam and tobacco Ginned cotton, yarn and cloth are the chief exports to Madras and the southern districts Tea and coffee from the Nilgiris are exported to almost every place in India Paddy, grain and miscellaneous articles form the chief imports from the west coast A small quantity of cotton is imported from Bombay

Plague—The history of plague in Coimbatore dates back to 1903 There is no record of how infection was introduced, but it was probably from Mysore State Plague first started in October 1903 The number of cases is not known 1904 and 1905 were comparatively free and there were a few imported cases from 1906 to 1909 Severe epidemics started in 1910 and every successive year plague raged in a very severe form and has been responsible for more than 8,000 attacks and 5,000 deaths from 1910 to 1924 In 1924 there were only eight attacks and deaths and since then there have been *no cases* and the place has been remarkably free from plague This freedom requires explanation Possibly it is the result of an extensive municipal rat-trapping campaign which has yielded about 21,000 rats a year since 1922 and which appears to have definitely diminished the number of rats

Rodents—*See* Table IV Only 325 *R rattus* and 6 bandicoots were trapped during the survey This is explained by the rat-trapping campaign mentioned above The *R rattus* were mostly brown-bellied, only 66 specimens of white-bellied rats being found The cotton seed godowns above showed a high rat density The bazaar, residential quarters and the cotton mills had comparatively low rat densities As usual, the percentage of female rats was larger than that of males

Fleas—*See* Table IV During the survey, all the three species of *Xenopsylla* were found and these were present in all the localities of the town *X cheopis*, however, is the prevailing flea of Coimbatore, next comes *X brasiliensis* and lastly *X astia* The cotton mills showed a very high general flea index of 13 56 and a specific *cheopis* index of 12 81 The bazaar and cotton seed godowns come next with general flea indices of 6 31 and 6 23 The *cheopis* indices for the same localities are 3 22 and 1 82 respectively

It should be noted that out of 570 fleas examined from the cotton mills 538 were *cheopis* and only 12 *astia* and 20 *brasiliensis* The cotton seed godowns show a higher index for *brasiliensis* (3 64) than for *cheopis* (1 82) The rice godowns which show a general flea index of only 4 20 show a higher *cheopis* index than the cotton seed godowns

TABLE IV

Rodents and fleas–Coimbatore (January-February 1930)

Locality	Number of traps laid	Number of rats trapped	Rat density (number of rats per 100 traps)	Total fleas	General flea index	*X astia*	*Astia* index	*X brasiliensis*	*Brasiliensis* index	*X cheopis*	*Cheopis* index
Bazaar	225	83	37	533	6·31	101	1·09	163	2·00	269	3·22
Rice godowns	30	5	17	21	4·20	2		8	1·60	11	2·20
Cotton seed godowns	15	17	113	107	6·23	14		62	3·64	31	1·82
Rice mills	15										
Cotton mills	150	42	28	570	13·56	12		20		538	12·81
Ginning presses	45	1	2	4						4	
Railway goods-shed	20	6	30	3				1		2	
Residential areas	575	177	31	819	4·45	81		277	1·54	461	2·61
TOTAL	1,075	331	31	2,057	6·10	210	0·52	531	1·59	1,316	3·99

Percentage of female rats	65·25	Percentage of *astia*	10·2
Percentage of pregnant rats to total females	25	Percentage of female *brasiliensis*	31·6
Average number of fœtuses	5·5	Percentage of *brasiliensis*	25·8
Replenishment rate for 100 rats per day	5·6	Percentage of female *cheopis*	36·1
Percentage of female *astia*	62·8	Percentage of *cheopis*	64

TABLE V

Loose flea data–Coimbatore (January-February 1930)

Locality	Rat density	*Astia* index	*Brasiliensis* index	*Cheopis* index	Ratio *cheopis* to other fleas	Number of 'de-fleaed' rats used	'Loose' *astia*	'Loose' *brasiliensis*	'Loose' *cheopis*	Ratio 'loose' *cheopis* to other 'loose' fleas
Cotton mills	28	12/42	20/42	12 81	16 8/1	78	2	2	31	8·5/1
Rice godowns	17	2/5	1 60	2 20	1 1/1	30			2	2·0/0
Cotton seed godowns	113	14/17	3 64	1 82	0·41/1	30	2	7	13	1 1/1

Loose flea observations—During the survey, 'loose flea' experiments with 'de-fleaed' rats were carried on in cotton mills and rice and cotton seed godowns The results are given in Table V The great number of loose *cheopis* in cotton mills and cotton seed godowns as compared with the paucity of loose fleas elsewhere is noteworthy

SUMMARY

(1) Three hundred and thirty-one rodents were trapped, 6 bandicoots and the rest *R rattus* The high rat density in cotton seed godowns alone with a low density elsewhere is noteworthy

(2) Two thousand and fifty-seven fleas were examined, out of which 10·2 per cent were *X astia*, 25 8 per cent were *X brasiliensis* and 64 per cent were *X cheopis* *X cheopis* is the commonest rat flea of Coimbatore and is particularly prevalent in the cotton mills

(3) There were severe epidemics of plague from 1910–1924 since when the town has been remarkably free This present immunity is probably associated with the effective rat campaign that is being carried on

REPORT NO XIII

COCHIN, CALICUT AND MANGALORE ON THE WEST COAST

(A) BRITISH COCHIN AND MATTANCHERRY * (January 1930)

British Cochin is the headquarters of Cochin taluk in Malabar district It is a municipal town with a population of 20,637 The town is built on a narrow strip of land between the back-water and the mouth of the Cochin river It is the most important seaport on the west coast of India south of Bombay

Mattancherry, in Cochin State, is continuous with British Cochin It is also a municipal town with a population of 24,664 Commercially it is as important as British Cochin Almost all the grain godowns are situated here They have much back-water traffic with Ernakulam and Alleppy

Climate—The place is low and swampy and so the climate is very damp The mean annual temperature is 80°F and is very uniform throughout the year varying from a monthly average at 8 A M in 1929 of 75°F in January to 84°F in May Even during the dry season of the year, the air is very humid, owing to frequent showers The average annual rainfall is 113 3 inches, of which 73 05 inches fall during the south-west monsoon from June to September The average meteorological conditions, as observed during the survey, are given below —

Period of survey–1st January to 21st January, 1930

Mean dry bulb temperature at 9 A M 80 3° Fahr
" wet bulb temperature at 9 A M 76 5° Fahr
" relative humidity at 9 A M 84 per cent
" saturation deficiency at 9 A M 0 18 inches

*These two places were surveyed by Dr P V George

Commerce—Cochin imports large quantities of rice from Rangoon, Akyab, Cocanada and Calcutta Wheat, barley and pulses are imported from Bombay and Karachi The chief exports are pepper, copra, coco-nut oil and coir

Plague—Apart from two imported cases in 1928, British Cochin has not had plague Mattancherry had two small epidemics of plague, one in 1919 with 20 deaths and the other in 1928 with 14 deaths Both these epidemics were in the rainy months of June, July and August, which are also the coolest months

During both these epidemics rat-falls commenced two weeks earlier, and continued to occur in 1919 for two months and in 1928 for over 4 months after the termination of the epidemic Further, it appears that the number of rat-falls was unusually great considering the number of human cases In 1928, 659 rat-falls were officially recorded in Mattancherry and a few in British Cochin also, but it would appear that this number is only a small fraction of what actually happened Bacteriological examinations done by the Health Department of Cochin State were positive in 42 per cent of the rats examined Local inquiry also shows that practically every year rat-falls are of common occurrence but are not accompanied by human cases Even during the period of this survey there were a few rat-falls in Mattancherry So rat epizootics seem to be common, while human plague on the other hand is infrequent

The survey was done in January 1930 All representative areas were done and also an island off British Cochin

Rodents—One hundred and sixty-nine rodents were trapped, out of which 127 were *R rattus*, 34 *R norvegicus*, 7 musk-rats and 1 bandicoot

Although most of the specimens of *R norvegicus* were obtained in the godown areas in Mattancherry and Kalvetty Bazaar, yet they were also obtained from places away from trade centres and from Vypen, an island opposite British Cochin

From Table I we may note that the rat density was uniformly low in all areas, except Mattancherry Bazaar This low density cannot be explained by any rat catching done locally It is probably due to the frequent rat epizootics of plague or some other disease, that occur here

All rodents obtained were autopsied In two specimens, scars of healed abscesses were noticed on the spleens All spleen smears were negative for *B pestis*

Rat-fleas—Four hundred and thirteen fleas were gathered and of these 341 were *X astia*, 71 *X brasiliensis* and 1 *Ctenocephalus felis*

Table I gives the flea indices, etc, for the different places The flea indices were uniformly low in all the areas, ranging from 1 5 in residential areas to 3 in Amaravathy Bazaar

X brasiliensis was obtained only in the bazaars of Mattancherry, Kalvetty, and Amaravathy, and these are the areas where the grain godowns are all situated This flea was conspicuously absent from the extensive godowns in British Cochin, which store export materials such as pepper, copra, coir, etc.

TABLE I

Rat-flea survey–Cochin and Mattancherry (January 1930)

Place	Traps	Rats	Rat density (number of rats per 100 traps)	Total fleas from all rats	Total flea index for *R rattus*	Total *X astia* from all rats	*Astia* index for *R rattus*	Total *brasiliensis* from all rats	*Brasiliensis* index for *R rattus*	REMARKS
British Cochin										
Kalvetty Bazaar	51	14	27	35	2·7	33	2·5	2	0·2	Area with godowns for imported grains
Amravathy Bazaar	51	10	20	30	3·0	18	1·8	12	1·2	
Pepper, copra and coir godowns	102	17	17	12	1·1	12	1·1			
Retail bazaar areas	102	18	18	53	2·9	53	2·9			
Residential areas	207	27	13	85	1·5	85	1·5			
Vypen island	102	21	21	30	1·5	29	1·5			
Mattancherry										
Bazaar	102	56	55	157	2·5	101	1·3	56	1·2	Area with godowns for imported grains
Residential area	102	6	6	11	2·0	10	1·8	1	0·2	
TOTAL	819	169	21	413	2·1	341	1·7	71		

Percentage of female rats	65	Replenishment rate of 100 rats per day	3
Percentage of pregnant female *R rattus* to total females	13	Percentage of female *X astia*	50
Average number of fœtuses per pregnant female	5·2	Percentage of female *X brasiliensis*	39

In British Cochin *brasiliensis* fleas were obtained only in the premises of godowns situated close to Mattancherry

The specific *astia* index of *R rattus* was 2 15, while that of *R norvegicus* was 3 92 In *brasiliensis* areas, the specific *brasiliensis* index for *R rattus* was 0 9 while that of *R norvegicus* was 1 0

The lowness of the flea indices which is common to all areas is to be noted The total absence of *cheopis* has to be noted Since there have been a few cases of plague in Mattancherry as recently as 1928, when it is very unlikely that *cheopis* was present, we conclude that either *astia* or *brasiliensis* was the vector

Epizootics not plague—The most striking feature of plague in Cochin is that the epizootic was out of all proportion to the epidemic The reason is not obvious The rat-falls were more numerous in Mattancherry Bazaar and this is also the area where *brasiliensis* fleas prevail This suggests either that *brasiliensis* is a more efficient vector for rat plague than for human plague or that there was some other epizootic present not communicable to man There is some evidence for the latter in the report on Calicut

Summary

(1) Of 169 rodents 127 were *R rattus*, 34 *R norvegicus*, 7 musk-rats and 1 bandicoot The rat density was low in all areas, except Mattancherry Bazaar

(2) Of 413 fleas collected 341 were *X astia*, 71 *X brasiliensis* and 1 *Ctenocephalus felis* The flea indices were very low in all areas

(3) Epidemics of plague in the past were probably caused by *X brasiliensis* or *X astia* or by both

(4) The rat epizootics prevalent need further investigations

(5) Considering the increasing trade of this important port, and the risk of exporting infection, sustained plague preventive measures such as relief of housing congestion, improved sanitation and rat destruction are indicated

(B) Calicut* (January-February 1930)

Calicut, the headquarters of Malabar district, is a municipal town with a population of 82,334 It is an important trade centre, being on the main line of the South Indian Railway Its sea-borne trade is hampered by the absence of a sheltered harbour

Climate—The temperature is extraordinarily uniform, the mean monthly temperature at 8 A M in 1929 varying from 76°F in January to 85°F in May The average annual rainfall is 118 45 inches, of which about 89 inches fall during the south-west monsoon from June to September The average percentage humidity is high—82 5—varying from 90 per cent in June, July and

*This place was surveyed by Dr P V George

August, when the monsoon prevails, to 72 per cent in January The observations made during the period of survey were as follows —

Period of survey–22nd January to 18th February, 1930

Mean dry bulb temperature at 9 A M 80 6° Fahr
" wet bulb temperature at 9 A M 72 2° Fahr
" relative humidity at 9 A M 70 per cent
" saturation deficiency at 9 A M 0 3 inches

Housing and sanitation—Housing conditions seem to have a bearing on the endemicity of plague, for while the chief commercial areas like Big Bazaar Street remain practically free from infection, it is found that year after year plague occurs in certain low and middle class residential areas like Vellayil, Chettiada, Kappakal, Kaniamukuparambu, Annakulam, etc The houses in these crowded areas are simply huts with mud floors and palm-thatched roofs, standing in small gardens or coco-nut topes without any order They are poorly lighted and ventilated, and the prevailing conditions are ideal for both rats and fleas Their inhabitants are mostly fishermen

Commerce—The chief exports are tea, coffee, pepper, copra, coir, coco-nut oil, etc, products of the Nilgiri and other hills and of the adjacent coast lands The chief imports are grains and pulses from Bombay, Rangoon and Bengal, and raw cotton from Coimbatore, Bombay, etc

Plague—Calicut had plague first in 1907 and then for the next 10 years the town was free The infection reappeared in 1921 and has occurred *every year* since then During this survey plague was epizootic and epidemic Out of 480 autopsied rats, two showed caseating glands in the groin, smears from both showing *B pestis* Spleen smears from five other trapped rats also showed *B pestis*, of these four had been trapped in Vellayil, the chief plague focus of the town During the survey 14 dead rats picked up by the municipal plague staff were examined and the spleen smears of five showed *B pestis*

The monthly figures for attacks from plague for the last two years are as follows —

Year	January	February	March	April	May	June	July	August	September	October	November	December	Total
1928	48	20	21				8	5	15	16	10	15	158
1929	14	5	5	2				5	7	5	2	2	47

Thus plague appears to be practically continuous in Calicut, the only break that occurs is the short one due to the hot weather in April, May and June

Survey—The survey was done in February 1930 All representative areas in the town were surveyed

Rodents—Four hundred and eighty rodents were trapped and of these 450 were *R rattus*, 28 *R norvegicus* and 2 were bandicoots.

The distribution of *R norvegicus* was found practically limited to Big Bazaar Street, which commences from the beach and runs right across to the centre of the town. It is to be noted that this street contains all the godowns for imported grains, suggesting, what we already know on other grounds, that *R norvegicus* has been imported.

Table II gives the rat density for the different areas in the town. It is seen that the rat density is not very high in any of the areas, and as usual bazaar areas show higher densities than others. One good reason for the low rat density is the epizootic of plague. But there is some evidence that an epizootic, as yet unidentified, that is not plague, has also been at work in killing rats. This will be discussed later.

Among *R rattus* 64 per cent were females, and out of these 14·8 per cent were pregnant. This comparatively low pregnancy rate may be accounted for by the season. The replenishment rate was correspondingly low—(3). The rainy season generally shows a higher rate.

Rat-fleas—Out of 2,885 fleas gathered, 919 were *X astia*, 1,661 *X cheopis*, 205 *X brasiliensis*, 6 *Ctenocephalus felis* and one *Pulex irritans*.

Among the 3 species of *Xenopsyllæ*, *astia* and *cheopis* were obtained from all areas, while *brasiliensis* were only obtained from the Big Bazaar Street, evidently pointing to its importation from the hinterland with grains.

Table II shows the general and specific flea indices for *R rattus* and *R norvegicus*. As is usual, the latter harboured more fleas, the general index being more than three times the former.

The general flea index was fairly uniform in all areas, with two exceptions, Big Bazaar Street and Kallar Cotton mill, which were two and three times the average respectively.

In all three species of *Xenopsyllæ*, the males predominated, but more markedly with *brasiliensis*.

An epizootic, not plague—The evidence for this is as follows—

(1) During 1929, 430 rat-falls were recorded in Big Bazaar Street *alone*, while only 82 rat-falls were recorded from all the rest of the town. On the other hand, in this year only one human plague-death was traced to Big Bazaar Street, while 33 deaths occurred in the rest of the town. Thus there would appear to be some disproportion between rat-falls and human plague cases in the two classes of areas (1) the godown and bazaar areas where there are many rat-falls but little plague, and (2) the residential areas such as Vallayil where there are a few rat-falls and nearly all the human plague.

(2) During the survey, in spleen smears from 6 autopsied rats, some stout, rod-shaped bacilli, without any definite bipolar staining, were observed. Similar organisms were also found in 7 out of 14 dead rats examined during the same period. These bacilli were probably not putrefactive organisms since they were found even in rats which were examined from one to two hours after death.

TABLE II

Rat-flea survey–Calicut (January-February 1930)

Places	Number of traps laid	Number of rodents obtained	Number of rodents per 100 traps	Number of fleas obtained	Total flea index for *R rattus*	Number of *X astia* obtained	*Astia* index for *R rattus*	Number of *X brasiliensis* obtained	*Brasiliensis* index for *R rattus*	Number of *X cheopis* obtained	*Cheopis* index for *R rattus*
Big Bazaar Street (Grain godowns)	270	78	29	835	9·4	463	6 0	205	1 3	167	2·1
Retail bazaars	249	97	39	420	4 3	110	1 1	0		310	3 2
Godowns in New Customs Road (copra, coir and pepper godowns)	178	38	21	184	4 8	44	1 1	0		140	3 7
Nadakavu and West Hill	122	19	16	103	5 4	84	4 4	0		19	1 0
Residential areas	1,175	237	20	1,056	4 4	218	0 9	0		831	3·5
Kallai Cotton mill	42	11	26	194	17·6	0		0		194	17 6
TOTAL	2,036	480	24	2,792	5 4	919	1 5	205		1,661	3·5
R norvegicus		23		419	18·2	234	10·1	136	5 9	49	2·1

Percentage of female *R rattus*	64	Replenishment rate of 100 rats per day	3
Percentage of pregnant	14 8	Percentage of female *astia*	48
Percentage of pregnant to total rats	9 4	Percentage of female *brasiliensis*	30
Average number of fœtuses per pregnant female	5 1	Percentage of female *cheopis*	40

Crows were dying in large numbers in the town, and in two specimens examined, the smears from the throat, spleen, etc, showed various bacilli, some of which appeared morphologically similar to the bacilli found in rats In one spleen smear from a captured autopsied rat in Cochin similar organisms were seen

No further examination was done and so no definite conclusions can be reached, but since rat epizootics are important as a means of controlling plague, this epizootic will probably be worth further investigation A rat epizootic like plague (D'Aar disease) has already been reported in Africa by J B Mitchell in the *Journal of Hygiene* of February 1930

SUMMARY

(1) Four hundred and eighty rodents were trapped, 450 were *R rattus*, 28 were *R norvegicus* and 2 bandicoots *R norvegicus* was caught only from Big Bazaar Street

(2) Two thousand eight hundred and eighty-five rat-fleas were identified, 919 were *X astia*, 1,661 *X cheopis*, 205 *X brasiliensis*, 6 *Ctenocephalus felis* and 1 *Pulex irritans*

The specific *cheopis* index for Kallar Cotton mill was about five times the average *X brasiliensis* fleas were obtained only from godowns in the grain trade area

(3) There is some evidence for the existence of an epizootic fatal to rats that is not plague

(4) Plague has been endemic for the last several years, and all factors present favour its continuance More vigorous and sustained preventive measures are indicated particularly in the improvement of housing conditions and sanitation

(c) MANGALORE* (February-March 1930)

Mangalore, the headquarters of south Kanara district, is a municipal town with a population of 53,877 It is an important seaport trading with many small ports on the west coast, and with Bombay, Colombo and Rangoon, except during the south-west monsoon when sea traffic practically ceases It is also the terminus of the South Indian Railway in the west coast of the presidency The town is surrounded on three sides by water, being situated on the back-water formed by the convergent mouths of two rivers

Climate—The town enjoys a fairly uniform temperature resembling Calicut The mean monthly temperature at 8 A M in 1929 varied from 76°F in January to 84°F in May The average annual rainfall is about 133 85

*This place was surveyed by Dr P V George

inches, of which about 113 96 inches fall from June to September The average meteorological conditions as observed during the survey are as follows —

Period of survey–19th February to 9th March, 1930
Mean dry bulb temperature at 9 A M 81 5° Fahr
„ wet bulb temperature at 9 A M 74 0° Fahr
„ relative humidity at 9 A M 71 per cent
„ saturation deficiency at 9 A M 0 33 inches

Commerce—The chief imports are grains from Bombay and Rangoon and cotton yarn The chief exports are coffee grown in Mysore and Coorg, areca nuts, cardamoms, sandalwood oil, tobacco, bricks and tiles

Housing and sanitation—Most of the houses have tiled roofs and tiled floors The sanitary conditions are good and the whole town present a picturesque and clean appearance

Plague—Plague first occurred in 1902, and except in 1920 and 1921, *reappeared yearly* up to 1927 causing 169 deaths a year on the average Every part of the town had plague in one or other of these years The town has been completely free from plague for the last three years Why, it is not apparent Rat catching has been going on from 1902 onwards In September 1925 a few cases of pneumonic plague occurred Figures for seasonal prevalence are not available, but according to Lieut-Col A J H Russell's report on the geographical distribution of plague in the Madras Presidency, the plague curve for the district of south Kanara is similar to that of Malabar The lowest death rate, 0 03 per mille, occurs in June and after an increase to 0 13 per mille in September, a slight fall is noted in October and November, soon followed by a rise to the low maximum of 0 21 in March

The survey was done in February-March 1930 All representative areas and also a small fishing, sea-coast village, Thanneerbhavi, were surveyed, and the data have been tabulated for the different types of area

Rodents—Four hundred and two rodents were trapped, of these 351 were *R rattus*, 28 house mice and 23 musk-rats

Table III shows the rat density, etc, for the different areas Despite the continued rat catching (16,000 last year), the rat densities are just about the average

Rat densities were highest in the commercial areas Female rats predominated in all areas, and the replenishment rate for 100 rats per day was found to be 4 7. Musk-rats and house mice abounded

All rodents obtained were autopsied and spleen smears examined with negative results for plague

Rat-fleas—Two thousand and fifty-one rat-fleas were examined, of these 482 were *X astia*, 396 *X brasiliensis*, and 1,170 *X cheopis* and 3 *Ctenocephalus felis*

Table III gives the flea indices for *R rattus* in the different areas It is to be noted that here, just as in Cochin and Calicut, the distribution of

TABLE I

Rat-flea survey–Mangalore (February-March 1930)

Places	Number of traps laid	Number of rats obtained	Number of rats per 100 traps	Total fleas gathered	Total flea index for *R rattus*	Total *X astia* gathered	*X astia* index for *R rattus*	Total *X brasiliensis* gathered	*X brasiliensis* index for *R rattus*	Total *X cheopis* gathered	*X cheopis* index for *R rattus*
Gollikatta Ward (grain godowns)	447	185	41	1,169	6·9	349	2·1	395	2·3	423	2·5
Retail bazaar area (Market Bazaar and Car Street)	131	68	52	159	2·8	40	0·7	1		118	2·1
Coffee stores	54	11	20	39	3·5					30	2·7
Fishermen's huts	117	43	37	251	7·0	14	0·4			237	6·6
Middle class residential areas	348	90	26	409	4·9	65	0·8			343	1·1
Thanneerbhavi village	50	5	10	24	6·0					19	4·7
TOTAL	1,147	402	35	2,051	5·7	468	1·3	396	1·1	1,170	3·3

Percentage of female *R rattus*	74	Replenishment rate of 100 rats per day	4·7
Percentage of pregnant female *R rattus*	22	Percentage of female *astia* fleas	53
Percentage of pregnant female *R rattus* to total	16	Percentage of female *brasiliensis* fleas	38
Average number of fœtuses	4·7	Percentage of female *cheopis* fleas	38

X brasiliensis was limited to the wholesale grain trade area comprised of Gollikatta Bazaar Street, Bundu Street, Port Road, Alimuchi Bazaar and Kakidi Bazaar It is curious that the Market Bazaar, and the Car Street which form the chief retail bazaar areas, showed only one *brasiliensis* flea in 159 fleas

Cheopis was the predominating flea in all areas except in Gollikatta Ward where *brasiliensis* also prevailed in almost equal proportions *Cheopis* was also the chief flea in Thannerbhavi village, which is separated from Mangalore town by a broad river

As seen from the table, the flea indices, especially the *cheopis* index, were fairly high in all areas From 21 musk-rats caught alone in traps, 78 fleas were collected, giving an *astia* index of 0 38 and a *cheopis* index of 3 3

Summary

(1) Four hundred and two rodents were trapped, 351 were *R rattus*, 28 house mice and 23 musk-rats

(2) Two thousand and fifty-one rat-fleas were gathered, of these, 482 were *X astia*, 396 *X brasiliensis*, 1,170 *X cheopis* and 3 *Ctenocephalus felis*

The highest *cheopis* index was found in the insanitary fishermen's huts in the town The distribution of *cheopis* and *astia* was general, while that of *brasiliensis* was limited to the areas of wholesale trade in imported grains

(3) In addition to carrying on the rat campaign with vigour it would be of distinct benefit to rat-proof godowns

CERCARIÆ NICOBARICÆ *

BY

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INTRODUCTION

DURING the winter months of 1921-22 the R I M S 'Investigator' was engaged in a survey of Nankauri Harbour in the central group of the Nicobar Islands, and the opportunity was taken of collecting and examining for trematode parasites such fresh-water molluscs as could be found in the neighbourhood Owing to the general configuration of the islands any permanent collections of purely fresh water are few and far between, four such areas, however, were found and investigated, namely —

(1) A small stream flowing into Nankauri Harbour near Ray Point in Camorta Island,

(2) A small muddy pool near the edge of a mangrove swamp on the margin of Back Bay on the east side of Camorta Island,

(3) An artificial lake, made by throwing up a 'bund' or dam across a small ravine on the north side of the old settlement at the south-east end of Camorta Island, and

(4) A marshy area behind a grove of palm trees about half a mile to the north of Naval Point, on the east side of Camorta Island

The species of fresh-water molluscs that were obtained from these localities were —

Melanoides nicobarica Morch,
Melanoides creberi (Reeve), and
Neritina zigzag (Lam.) Sow

The only species that was found to be harbouring parasites was *Melanoides creber* (Reeve) and from this single species of mollusc no less than five species

* A monographic study of Indian cercariæ by Major Sewell was published as a supplementary volume in this *Journal* (Vol X, *Cercariæ indicæ*)

of trematodes and in one instance infection with an indeterminable sporocyst were discovered The rate of infection by the different species of trematode in the different months during which I was able to make observations is given in Table I

In one instance a double infection, by *Cercariæ nicobaricæ* I and IV, was encountered in the month of December

TABLE I

Showing the distribution of infection in Melanoides tiebei (*Reeve*) *in each month, during which observations were made*

Month	*Cercaria nicobarica* I	*Cercaria nicobarica* II	*Cercaria nicobarica* III	*Cercaria nicobarica* IV	*Cercaria nicobarica* V	Indeterminate sporocysts	Uninfected	Total number examined	Percentage infected	Locality
November 1921	0	1	1	1	1	1	2	7	71·4	1
December 1921	1	2	0	5	2	0	9	18	50·0	1
January 1922	0	0	14	0	0	0	7	21	66·7	2
TOTAL	1	3	15	6	3	1	18	46	60·87	

THE 'PLEUROLOPHOCERCA' GROUP

Cercaria nicobarica I, sp nov

(Plate XLVI, fig 1)

This cercaria was somewhat sluggish

The body in a state of moderate extension measured 0 384 mm in length and 0 123 mm in breadth, the tail was long and tapering and measured 0 439 mm in length The body was narrowly elongate having its greatest width about one-third of its total length from the anterior end, from this point it tapers to a rounded anterior extremity, the extreme anterior part, containing the oral sucker, being in certain stages of contraction sharply demarcated by a transverse groove Posteriorly the body is truncated The surface of the body is armed throughout the anterior two-thirds of its length with numerous small backwardly-directed spines that are set in transverse rows There is no trace of any acetabulum or of lateral locomotor pockets The tail is attached on the ventral aspect of the posterior end of the body and is about two-thirds of the width of the body at its base One-third of its length from the base a delicate fin-fold commences in the mid-dorsal line and is continued back around the

extreme tip of the tail, where it becomes continuous with a ventral fold that can be traced forwards for about one-third of the length of the tail This fin-fold shows a number of fine 'rays' resembling setæ, but these appear to me to be due to the folding of the delicate membrane and not to any definite supporting structures At its base well-marked longitudinal muscle fibres can be seen passing backwards in the substance of the parenchyma Unlike the closely allied monostomes, *Cercariæ indicæ* VII and VIII, there was no pigment in the body and I was unable to detect any eyes, even unpigmented

The anterior region of the body is occupied by a rounded oral sucker, the anterior lip of which is provided with a double row of minute highly refractile spines I was unable to detect any mouth or pharynx The central portion of the body is occupied by a conspicuous mass of pyriform salivary cells, having granular protoplasm and round clear nuclei There were in all fourteen such salivary cells From these ducts could be traced forwards to the posterior margin of the oral sucker, and they probably open near the row of spines mentioned above

Lying in the posterior half of the body behind the salivary cells is a U-shaped mass of small round granular cells, these I take to be the cells covering the excretory bladder The excretory orifice is situated on the dorsal aspect of the point of attachment of the tail I was unable to detect any excretory tubules or flame cells

Development occurs in sausage-shaped rediæ that vary in size from 0 507 mm to 0 740 mm in length and from 0 110 mm to 0 164 mm in breadth In young immature rediæ the anterior end is occupied by a rounded pharynx and this is followed by a long saccular stomach that reached back to about one-half of the total body-length In mature rediæ the pharynx is oval in shape and measures 0 064 mm in length by 0 048 mm in breadth All trace of any stomach has completely disappeared in the largest mature forms Even in the small examples in which a stomach is present it is for the most part devoid of any contents The cercariæ almost certainly leave the rediæ before they are fully mature No mature cercariæ were seen in any redia, though in all the larger examples several immature specimens were present and numerous mature or nearly mature cercariæ were found free in the substance of the liver of the mollusc host The mollusc host in which this cercaria was developing was *Melanoides creber* (Reeve) and the infected individuals exhibited a liver of an unhealthy grey colour Examples were taken in the month of December 1921 from a small stream at Ray Point, Camorta Island, Nicobars

This cercaria clearly belongs to the 'Pleurolophocerca' group of the monostomes It is very closely allied to *Cercariæ indicæ* VII and VIII but differs from both in the complete absence of eyes

The 'Pleurolophocerca' group was created by me in 1922 in order to include a number of species that possess the following characters (1) the presence at the anterior end of the body of a protrusible organ, designed for penetration rather than adhesion and armed with penetrating spines, (2) conspicuous

paired salivary glands, composed of several cells, the ducts of which pass forward to open on the penetrating organ, (3) the excretory bladder is reniform or trifoliate in shape and possesses thick walls, (4) the tail is long and powerful and is provided with cuticular fringes or fins, (5) there are no locomotor pockets at the postero-lateral regions of the body, and (6) development occurs in rediæ In my original account I gave, as a further character, the absence of an acetabulum The work of Langeron* and Dubois (1929, p 37) on *Cercaria pleurolophocerca* Sons and *C lophocerca* Fil has rendered a modification of this statement necessary and Dubois suggests the addition of the following (7) an acetabulum may exist, well developed (*Cercaria pleurolophocerca*) or much reduced (*Cercaria lophocerca*) or may have completely disappeared (*Cercariæ indicæ* VII and VIII)

In this group I then placed the following species —

Cercaria pleurolophocerca Sons,
Cercaria lophocerca Fil,
Cercaria lophocerca Lebour (*nec* Fil),
Cercaria indica VII, and
Cercaria indica VIII

I also provisionally included *Cercaria indica* III, although this species exhibits certain differences of structure To this group must also be added *Cercaria quadripterygia* Ssmitzin (1911), which appears to agree in all essentials with the other members of the group, and *Cercaria parvomelanæ* Tubangui (1928)

Dubois (1929, p 141) has pointed out that these species do not form a true group, but are more of the nature of a series in which one can trace a gradual reduction of the acetabulum The discovery of *Cercaria nicobarica* I, in which the above characters are present while certain others are absent, still further extends this evolutionary series

XIPHIDIOCERCARIÆ

Cercaria nicobarica II, sp nov

(Plate XLVI, figs 2, 3*a*, 3*b* and 4)

The cercaria is small, the body-length being only from 0 086 mm to 0 138 mm, and the breadth varies from 0 037 mm to 0 068 mm The tail has a length of 0 108 mm and a breadth at its base of 0 018 mm The body is covered throughout with numerous minute backwardly-directed spines that are set in transverse rows and are distinctly larger at the posterior end on either side of the attachment of the tail The anterior end of the body is occupied by the rounded oral sucker that has a transverse diameter of 0 034 mm The

* Langeron, M, 1920, 'Recherches sur les Cercaires des Piscines de Gafsa et enquete sur la Bilharzoise tunisienne' *Archives de l'Institut Pasteur de Tunis* XIII (I have not been able to refer to this work—Author)

sucker is composed of parenchymatous cells and is only a very feeble prehensile organ, so feeble, indeed, that in the absence of an acetabulum, the cercaria appears unable to progress 'on the flat' The dorsal wall of the sucker is occupied by a stylet (Plate XLVI, figs 3*a* and 3*b*) The stylet has the usual thickening situated about one-fourth of its length from the anterior end and the thick wall terminates about one-fourth of the length from the posterior end, the extreme posterior portion being thin walled The dorso-ventral depth of the stylet is one-third of its length, the actual measurements being 0 018 mm in length by 0 006 mm in depth The stylet appears to be slender when viewed from the dorsal aspect but is very wide dorso-ventrally There is no acetabulum The tail is attached on the ventral aspect of the body a little way in front of the posterior margin and forms an angle with it, it is completely devoid of spines

The mouth appears to open near the base of the stylet and from it a short pre-pharynx passes back to a small rounded pharynx, behind this a longitudinal band of tissue passes backwards in the middle line to a small elongate cavity situated between the two anterior pairs of salivary gland cells

The salivary glands consist of three pairs of cells The anterior two pairs lie in apposition with each other in the middle line, they are relatively small and have finely granular protoplasm with clear round nuclei The posterior pair of salivary gland cells are large and conspicuous and lie behind and to the outer side of the other two pairs, they possess a crenated posterior and outer margin and are composed of coarsely granular protoplasm with refractile granules, each cell has a clear round nucleus From each cell a wide and conspicuous duct passes forwards and the three ducts appear to wind spirally round each other The duct of the posterior coarsely granular cell is disproportionately large and might be itself taken for an extra salivary gland cell

The three ducts become extremely attenuated at the side of the oral sucker and it was only with difficulty that I was able to trace them to their termination on either side of the stylet

Lying behind the oral sucker and in front of the salivary gland cells is a mass of hyaline substance, which appears to be made up of two large oval masses lying in contact with each other in the middle line behind the pharynx, this I take to be the rudiment of the brain

About one-fourth of the body-length from the posterior end is a mass of small round cells that probably is the rudiment of the future acetabulum Behind and around this is a mass of smaller cells that is probably the rudiment of the genital organs, and from this mass a prolongation passes forwards in the middle line

The excretory bladder lies a short distance in front of the posterior end of the body, it is small and has a reniform or U-shaped cavity From each antero-lateral cornu a main excretory duct arises and follows a convoluted course forwards As a result of the constant crossing and recrossing of the duct I found it extremely difficult to trace the course of the finer tubules, but it

seems probable that each main collecting tubule divides into two branches, the accessory collecting tubules Of these one passes forwards, and after giving off a branch that runs forwards and terminates in a series of capillaries in and around the oral sucker, turns back on itself and after sending branches to the brain terminates in the region of the genital organ, the other accessory tubule passes backwards and finally ends near the excretory bladder I was quite unable to detect any flame-cells, so that the exact arrangement of the whole system must for the present remain a matter of conjecture, but, judging from the arrangement of the tubules, the system is a complicated one

Development occurs in small oval sporocysts, measuring 0 080 mm in length by 0 040 mm in breadth Each sporocyst contained from one to five mature cercariæ and in addition a number of developing immature cercariæ and germ-balls

The mollusc host was *Melanoides crebei* (Reeve), and the liver of infected examples was of a grey colour Infected specimens of the host were taken in three different localities in Camorta Island

Examination of further examples of *Melanoides crebei* (Reeve) taken on 19th December, 1921, from a different locality, namely a marshy area lying behind a grove of palm trees about half a mile to the north of Naval Point on the east side of the island of Camorta, revealed the presence of sporocysts and cercariæ that very closely resemble the above form but yet differ in certain small details of structure So close is the resemblance, however, that I am unable to decide whether they are identical or represent a distinct but very closely related species

These latter forms (Plate XLVI, fig 4) are identical with the former as regards the size of the cercariæ, and the general characters of the bodily structure are also identical, with the following differences Behind the pharynx there runs in the middle line a narrow band, apparently of fibrous tissue, this passes back from the pharynx between the two brain masses and finally ends in a small elongate cavity situated between the anterior two pairs of salivary gland cells Behind this, extending backwards and outwards in a V, are three pairs of round clear vacuoles that I take to represent the rudiment of the intestinal cæca As in the other form, the salivary gland on each side of the body consists of three pairs of cells Of the salivary cells the anterior pair are small and are in close apposition with each other, being separated only by the rudiment of œsophagus The posterior pair are considerably larger and have crenated or wavy outer margins All three pairs of cells possess granular protoplasm, but the granules in the anterior two pairs are slightly larger than those of the posterior pair, thus exhibiting a condition the exact opposite of that present in the former type

The sporocysts of this type varied considerably in size and were rather larger than those of the former The length measurement ranges from 1 087 to 1 480 mm and the breadth from 0 348 mm to 0 522 mm In many of these sporocysts one end was produced in a small solid papilla with a rough shaggy

surface, as if this had served as a means of attachment to the liver substance of the mollusc host

As already mentioned the mollusc host was *Melanoides creber* (Reeve) and the percentage infection was 16 7 per cent or 1 in 6 Of these six specimens examined all were producing young, even the example infected with the trematode The liver of the infected individual was of a yellow colour, whereas the colour of the normal liver is dark brown with a tinge of green

The absence of a definite acetabulum in the present species is of considerable interest I have previously (*vide* Sewell, 1922) described two cercariæ, namely *Cercariæ indicæ* LII and LXI, that are clearly closely related to the Xiphidiocercariæ and yet differ from these distomes in possessing no acetabulum Both these monotome forms are characterized by —

(1) The possession of three to six pairs of salivary or penetration gland cells,
(2) the presence of a definite pharynx,
(3) the presence of a rounded or bicornuate excretory bladder, from which the main excretory tubes arise on each side and pass forwards to about the level of the middle of the body-length and there divide into anterior and posterior collecting tubes,
(4) the presence of a rounded and undifferentiated mass of small cells occupying the position, and presumably representing the rudiment, of the acetabulum, and
(5) development occurs in small oval or rounded sporocysts

In my previous paper (1922) I put forward the view that the central mass of small cells was the rudiment of the genital aperture, but in the light of further experience and the work of later authors it seems more probable that it is the rudiment of the acetabulum, the development of which has been delayed or suppressed

As I then pointed out, these two species, which I grouped together in the 'Ubiquita' group, appear to be closely related to certain Xiphidiocercariæ of the distome series in which the excretory system is of a simple type, although I was unable to trace the ultimate distribution of the capillaries or to detect the flame-cells In the possession of a definite pharynx and intestine, the presence of three pairs of salivary or penetration gland cells, and of a bicornuate excretory bladder the present form appears to come near to the 'Parapusilla' group of the Xiphidiocercariæ, in which I included *Cercariæ indicæ* V and XVI In both these forms there are three pairs of salivary gland cells, the most posterior of which possesses a crenated or lobulated margin, a well-developed pharynx followed by an œsophagus that terminated posteriorly in two short intestinal cæca and a bilobed excretory bladder, but it is probable that the excretory system is more complicated than in that group, in the members of which I was able to detect only six pairs of flame-cells, the arrangement of which may be represented by the formula $2[(1+1+1)+(1+1+1)] = 12$ flame-cells

Dubois (1929, p 72) has described two species of microcotylous cercariæ, *Cercaria helvetica* XI and *C helvetica* XII Although in the former he was able to detect only 9 pairs of flame-cells, while in the latter he found 12 pairs, he groups the two forms together in an additional group for which he proposes the name 'Helvetica' and gives as the excretory formula of the group 2[(2+2+2)+(2+2+2)[= 24 flame-cells, which, according to Faust (1924, Table II), is characteristic of the Dicrocœliinæ Neither of these new forms possess any trace of an œsophagus, although a well-developed pharynx is present Finally in the groups of the Xiphidiocercariæ that correspond to the subfamilies Plagiorchiinæ, Brachycœliinæ(?) and Mesocœliinæ, the excretory formula, according to Faust's table, is 2[(3+3+3)+(3+3+3)] = 36 flame-cells There is thus a progressive increase in the number of flame-cells in the various groups but to which group the present species is most nearly related must for the present be left undecided

THE 'MEGALURA' GROUP

Cercaria nicobarica III, sp nov

(Plate XLVII, figs 5 and 6)

A Megalurous cercaria that is very closely related to the form described by Sonsino under the name *Cercaria distomatosa* was taken during the month of January 1922

The cercaria swims by means of undulations of its body, the tail taking no part in the process and being dragged behind through the water, the direction of swimming is always upwards towards the surface, periods of activity alternating with periods of rest

The body is long and narrow the greatest diameter occurring at the level of the acetabulum The body-length varies from 0 69 mm to 0 342 mm and the breadth from 0 137 mm to 0 247 mm The surface of the body presents a granular appearance owing to the presence just below the cuticle of large numbers of cystogenous cells These cells tend to obscure the finer structure but I was quite unable to detect any spines on the surface of the body such as occur in the closely allied species *Cercaria indica* IV Sewell (*vide* Sewell, 1922, p 138, Plate XV), in this respect the present form agrees exactly with *Cercaria megalura* Cort and with Looss' account of *Cercaria distomatosa* Sons In *Cercaria indica* IV, as I have pointed out (*loc cit*, p 139), the surface spines become much more clearly visible after encystment but in the present form, even after this process had been completed, I was still unable to detect any spines on the surface of the body The tail is tapering and at the base has a diameter of 0 066 mm , its length varies from 0 397 mm to 0 493 mm , it is thus appreciably shorter than in *Cercaria indica* IV, in which it measures from 0 438 mm to 0 614 mm and still more so than in *Cercaria megalura* Cort, who gives the measurement in his examples as from one-half to ten times the length of the body The extreme tip of the tail is occupied by the characteristic

glandular organ and in this species there are ten cells present, whereas in *Cercaria indica* IV there are only four and in *Cercaria megalura* Cort there are from fifteen to twenty, in this respect the present specimens agree exactly with Looss' description of *Cercaria distomatosa* Sons The acetabulum is situated slightly behind the middle of the body-length, the proportions of the pre- and post-acetabular regions being 20 to 16 Its diameter is 0 089 mm

The anterior end of the body is occupied by an oval or rounded oral sucker, having a transverse diameter of 0 67 mm and an antero-posterior length of 0 82 mm

The mouth is situated at the anterior end in the centre of the oral sucker and leads back, through a short pre-pharynx, to a well-developed rounded pharynx, having a diameter of 0 040 mm from before backwards and 0 037 mm from side to side Two distinct regions can be recognized in the pharyngeal bulb, namely an anterior muscular zone and a posterior region composed of small round cells that may have a glandular function The pharynx is followed by a wide œsophagus that after a short course bifurcates into two long intestinal cæca, these run back to the posterior part of the body, terminating on a level with the anterior end of the excretory bladder I was unable to detect any salivary gland cells but a number of ducts passing forwards on either side of the pharynx could be traced to the anterior margin of the mouth where they open on the surface, the terminal part of each duct is marked by a short refractile body

The excretory system presents the same main characters as that of *Cercaria indica* IV The excretory vesicle is pyriform in shape and opens to the exterior by an excretory pore situated at the posterior end of the body on the dorsal aspect of the attachment of the tail From the truncated anterior end of the bladder two main excretory canals pass forwards to the level of the pharynx and then turn backwards till they reach the level of the acetabulum, in this second part of the tube there are situated three ciliated patches in which the cilia work forwards At the level of the acetabulum these canals bifurcate into anterior and posterior collecting tubules There are in all fifteen pairs of flame-cells in the body in the positions shown in Plate XLVII, fig 5 I was unable to trace all the connections but the anterior six pairs of flame-cells appeared to be connected with the collecting tubes in sets of three and it is probable that all fifteen are arranged in this manner In this respect the present species presents a contrast to the form previously described by me, *Cercaria indica* IV Sewell (1922, p 138, Plate XV, fig 3) In this latter form I could detect at least fourteen pairs of flame-cells in the body, though, as I then stated, I was never able to make out all the flame-cells in the same individual These fourteen cells appeared to be connected with the collecting tubules in groups of four in the anterior part of the body and in groups of three in the posterior region Further investigations in order finally to establish the basic pattern of the system in this group is eminently desirable I was able to trace a strand of tissue commencing from the posterior margin of the excretory bladder and

running for a short distance down the tail-stem, after which it divided into two strands which ran diagonally to the surface, this corresponds exactly in position with the caudal excretory canal in *Cercaria distomatosa* Sons, as figured by Looss and also shown by Manson-Bahr and Fairley, but I was unable to convince myself that it actually formed a patent canal

The genitalia consist of a mass of small round cells lying in front of the excretory bladder, which represents the ovary, and from this a column of cells passes forwards towards the acetabulum In front of the acetabulum and between the diverging intestinal cæcæ lies a second cell mass, the vagina or genital orifice I was unable to trace any complete cellular connection between these two cell masses, so that in this respect this form also agrees exactly with *Cercaria distomatosa*

The nervous system consists of two triangular cerebral ganglia or brain-masses, lying on each side of the pre-pharynx and connected together by dorsal and ventral commissures Anteriorly a pair of nerves, one from each ganglion, pass forwards to the sides of the sucker and a large nerve passes backwards laterally to the acetabulum for about two-thirds of the body-length

Development takes place in rediæ and there appears to be more than one generation of rediæ

A mature cercaria-producing redia (Plate XLVII, fig 6) measures from 1 945 mm to 1 575 mm in length and from 0 205 mm to 0 274 mm in breadth A pair of well-marked locomotor processes are present about one-fifth of the total body-length from the posterior end The mouth is situated terminally and is surrounded by thick, well-developed lips A birth pore is present but its position appears to be slightly variable, ranging from one-fourth to one-sixth of the body-length from the anterior end The body widens from the mouth to the region of the birth pore and the sides then remain more or less parallel till the region of the posterior locomotor processes behind which it tapers to a conical tail or may be more or less rounded with a blunt terminal process The anterior end of the redia is occupied by a well-developed pharynx, having a diameter of 0 062 mm This is followed by a long intestine that varies somewhat in length with the age of the redia, in immature rediæ the intestine reaches back as far as the posterior locomotor processes but in mature examples it only extends to a level about half-way between the birth pore and the locomotor process The intestine is filled with scattered orange or yellow granules The excretory system is well developed The main excretory tube opens about one-third of the total length from the anterior end It at first passes forwards and upwards for a short distance and then divides into two, the anterior and posterior collecting tubes The anterior collecting tube is very short and almost at once divides into two accessory tubes, each of which terminates in a single flame-cell The posterior collecting tube is long and passes backwards to a level behind the middle of the body-length before dividing into two accessory tubes each of which again ends in a single flame-cell There are thus four pairs

of flame-cells in the whole body and these are very large and closely resemble in character those found in the amphistomes

These rediæ were living in the upper part of the branchial chamber The liver of an infected snail was of a slate grey colour

The mollusc host of this species was *Melanoides creber* (Reeve) and the examples were taken from a small stream on the edge of a mangrove swamp on the east side of Camorta Island in the central group of the Nicobars The proportion of infected snails was six out of thirteen in the month of January 1922 A few days later more examples of the same mollusc also infected with the same cercaria were taken from a small muddy pool near Back Bay in Camorta Island, on this occasion every snail examined was found to be infected

Another example of the same species of *Melanoides*, namely, *M creber* (Reeve), from the same locality, was found to be infected with rediæ that were producing daughter-rediæ These rediæ agree in all essentials with the cercariæ-producing rediæ and are in all probability members of a previous generation

The total length of these rediæ varied from 0 753 mm to 1 726 mm and the breadth from 0 178 mm to 0 466 mm The mouth was terminal and was surrounded by thick fleshy lips The external surface of the redia differed in individuals of different degrees of maturity, in young examples the wall was thrown into low transverse ridges, whereas in older specimens it presented a shaggy appearance owing to the presence of numerous low processes A pair of well-developed locomotor processes are present about one-fifth of the total length from the posterior end The extreme posterior end is bluntly pointed

The mouth leads back into a well-developed pharynx that is either spherical or slightly oval in shape, in a specimen that had a total length of 0 753 mm the pharynx possessed a diameter of 0 068 mm and in one measuring 1 027 mm the pharynx measured 0 079 mm and 0 075 mm in the antero-posterior and transverse diameters respectively These figures demonstrate very clearly the small increase in size of the pharynx with the growth of the redia The pharynx is succeeded by a long intestine that reaches back nearly to the level of the posterior locomotor processes and a birth pore is situated at about one-fifth of the total length from the anterior end The excretory system appeared to be identical with that of the cercariæ-producing rediæ

These rediæ were living in the branchial chamber of the mollusc and were attached by their posterior ends, the anterior ends being free in the cavity and were continually moving from side to side

The manner in which these cercariæ encysted differed somewhat from that of *Cercaria indica* IV The cercaria swims upwards to the surface of the water by means of its body-movements only, the tail being dragged passively behind On reaching the surface the cercaria appeared to get a hold on the surface film by its oral sucker, and then drew up its body and obtained a similar hold on the surface by means of the acetabulum The cercaria then rests for a moment suspended in a horizontal manner from the surface film by means of its two suckers, the body being fully extended After a short pause the cercaria

lets go of its hold by the oral sucker and carries out a series of contractions of the body, the acetabulum being the centre of the movement As the body contracts a film of clear substance is left on the surface of the water, this substance appears to be the contents of the cystogenous cells that are extruded When the contraction of the body is completed, the animal secretes the usual bottle-shaped cyst around itself, the point where the acetablulum was attached to the surface film being left as a round hole The bottle-shaped cyst is thus slung, hammock-like, between the two floats that have been secreted by the anterior and posterior regions of the body respectively There is none of the rapid whirling movement that was seen in the case of *Cercaria indica* IV Sewell, and the tail appears to take no part on the cyst formation The dimensions of a fully-formed cyst are 0 248 mm in length and 0 123 mm in breadth

I have previously (Sewell, 1922, p 145) discussed the relationship of the various species belonging to this group of the cercariæ, which were known at the time of publication of my paper, and since that date the only additional account that has been published is that of Tubangui (1928, p 41, Plate II) who obtained what he believed to have been examples of *Cercaria indica* IV in the Philippines and to which he gave the name *Cercaria philippindica* The present species comes nearest to *Cercaria distomatosa* Sons, but as it differs in certain important particulars from the account given by Looss (1900, p 197, Plate XIV, figs 152 and 158) I prefer to regard it as a new species The chief differences are as follows —

(1) The average length of my examples appears to have been somewhat greater than Looss' specimens, but as his measurements were taken on preserved material it is as well not to lay too much stress on this point, but it is worth noting that the size of the two suckers is considerably greater, in *Cercaria nicobarica* III, the diameters are 0 082 mm and 0 089 mm for the oral sucker and acetabulum respectively, whereas in *Cercaria distomatosa* they are only 0 057 mm and 0 65 mm

(2) In the excretory system Looss (*loc cit* Plate XIV, 156) describes and figures a patent duct running back from the excretory bladder into the base of the tail-stem and then after a short course bifurcating to open on the lateral aspects, this is confirmed also by the observations of Manson-Bahr and Fairley (1921, Plate IV, fig 4) In the present examples I have been unable to convince myself of the existence of any such duct At the base of the tail there appears to be a solid strand of fibrous (?) material that takes its place

(3) In the redia the excretory system exhibits certain clear differences In the redia of *Cercaria distomatosa* the excretory orifice is placed rather far back in the lateral region and opens a short distance in front of the posterior locomotor processes and about one-third of the total length from the posterior end Each main collecting tube, after traversing an approximately equal distance, bifurcates into two accessory tubules and these again terminate in three flame-cells, so that there are in all twelve flame-cells on each side In

the present form the orifice lies considerably further forward and each accessory collecting tube ends in a single large flame-cell The difference in the number of flame-cells may be attributed to the age of the redia but it appears to be quite constant in all the rediæ examined by me and I am inclined therefore to regard it as a specific character

(4) In addition to these structural differences there are also differences in the general behaviour of the cercariæ, and especially in the mode of formation of the cyst

XIPHIDIOCERCARIÆ

Cercariæ microcotylæ

Cercaria nicobarica IV, sp nov

(Plate XLVII, fig 7)

(? *Cercaria pusilla* Looss, 1900, p 229, Plate XVI, figs 178–180)

The cercaria is a small one, measuring in total length about 0 25 mm The body varies in accordance with the degree of extension and contraction from 0 092 mm to 0 169 mm in length and from 0 046 mm to 0 077 mm in breadth The tail when extended has a length of 0 154 mm and a breadth at its base of 0 021 mm , when contracted it measures 0 084 mm in length by 0 022 mm in breadth The body, when extended, is elongate oval in shape, but when contracted and under cover-slip pressure it may become circular or even heart-shaped It swims slowly by contracting its body and curving it ventrally and then vigorously lashing its tail It crawls feebly by means of its two suckers The cuticle of the body appears to be devoid of actual spines, but under a high magnification possesses the appearance of numerous fine dots set in transverse rows

The anterior end of the body is occupied by a large oral sucker, circular in outline and having a diameter of 0 034 mm Embedded in the dorsal wall of the sucker is a well-developed stylet, that has a well marked thickening about one-fourth of its length from the anterior end, towards the posterior end of the stylet the diameter widens and the thick wall abruptly ceases, the stylet terminating posteriorly in a hemispherical thin-walled part The stylet only attains its final form in fully mature cercariæ, in immature forms it is of a simple needle-shape without the thickening round its anterior end The length of the fully-formed stylet is 0 018 mm

The acetabulum is situated at a distance of one-third of the total length from the posterior end It is much smaller than the oral sucker, having a diameter of only 0 015 to 0 018 mm It forms a well-marked protrusion on the ventral aspect, but does not appear to be a very powerful organ and seems to consist more of parenchymatous cells than muscle fibres

The tail is attached at the posterior end on the ventral aspect When fully extended the sides show a series of minute crenations, that probably correspond

to muscle-fibres, when contracted the anterior two-thirds of the tail is thrown into a series of folds but the posterior third seems to be but little affected The surface of the anterior two-thirds of the tail is smooth, the posterior third, however, is furnished with numerous very delicate hair-like spines

The mouth is sub-terminal and ventral and leads into the cavity of the oral sucker, its wall is lined with a thick layer of refractile substance, thus closely resembling the condition described by me in *Cercaria indica* V (*vide* Sewell, 1922, p 191, Plate XIX, fig 5) and figured by Looss (1900, Plate XVI, fig 179) in *Cercaria pusilla* Behind the oral sucker lies the rudiment of the pharynx, having a diameter of 0 011 mm I was unable to detect any other trace of an œsophagus or intestine The salivary glands consist of three cells, as is the case in most of the cercariæ belonging to this group Of these cells the anterior comes into contact with its fellow of the opposite side in the middle line in front of the acetabulum, the protoplasm is very transparent and contains numerous irregular masses of secretory material The middle pair of cells are pyriform in shape and possess refractile protoplasm with very fine granules scattered through it The posterior pair of cells are of a distinct brown tinge and have refractile, coarsely granular protoplasm, their posterior margins are lobulated Each cell sends a corresponding duct forwards to open on the antero-lateral wall of the mouth The anterior pair of cells and their ducts are very difficult to detect in the living cercaria and for some time I was under the impression that there were only two pairs of cells, but careful examination will reveal the third pair The great difficulty experienced in detecting the anterior pair of salivary gland cells and their ducts, combined with the marked brownish tinge of the posterior pair of cells, gives *Cercaria nicobarica* IV a striking resemblance to *Cercaria chlorotica* Dies and *Cercaria brunnea* Ercol Looss (*loc cit*) in his account of *Cercaria pusilla* does not mention the number of the gland cells He remarks, 'L'espace du corps compris en avant et aux côté de la ventouse ventrale est occupé par les glandes cephaliques, qui, au reste, n'offrent rien de special' In his figure (fig 179) he shows only two such gland cells, occupying the same positions in the body as the second and third cells in *Cercaria nicobarica* IV, but in front of and between these two cells he figures a number of coarse granules, he shows three distinct salivary ducts It thus seems probable that there are three pairs of salivary gland cells in *Cercaria pusilla* but that the anterior pair are difficult to detect, as in the present instance

The excretory bladder is U-shaped or reniform, depending on the degree of contraction From each antero-lateral cornu a main excretory tube can be traced forwards on each side of the body as far as the region of the acetabulum, where it divides into anterior and posterior collecting tubules, the course of the main duct is extremely convoluted and the point of bifurcation is very difficult to detect The anterior collecting tubule passes forwards and sends off a capillary that passes inwards and ends in a small flame-cell situated just in front of the salivary gland, the tube then continues forwards and after

bifurcation ends in a pair of flame-cells in the neighbourhood of the oral sucker The posterior collecting tubule could only be traced in its distal half, which passes backwards and ends in a pair of flame-cell on either side of the cornu of the excretory bladder A caudal excretory tube passes down the tail In the figure (Plate XLVII, fig 7) I have given the probable distribution of the whole system

The genital organs appear to be represented by a mass of round cells lying in the space between the diverging cornua of the excretory bladder and the posterior margin of the acetabulum

Development occurs in small sporocysts that are either round, having a diameter of 0 132 mm , or slightly oval, the two diameters of length and breadth being respectively 0 219 mm and 0 164 mm Each sporocyst contains two mature cercariæ and a number of developing germ-balls

The mollusc host was *Melanoides creber* (Reeve) and the example was taken from a small ravine on the south side of Flagstaff Hill, Camorta Island The bed of the stream was overgrown with weeds and grass and there was only a most meagre trickle of water coming down The liver of the infected snail was of a marked grey tinge

This cercaria undoubtedly belongs to the 'Microcotylæ' group of the Xiphidiocercariæ, and in its general appearance and characters comes near to the members of the 'Pusilla' sub-group It shows a very close degree of resemblance to *Cercaria pusilla* Looss and I give below for the purpose of comparison a table (Table II) showing the actual measurements in the two forms —

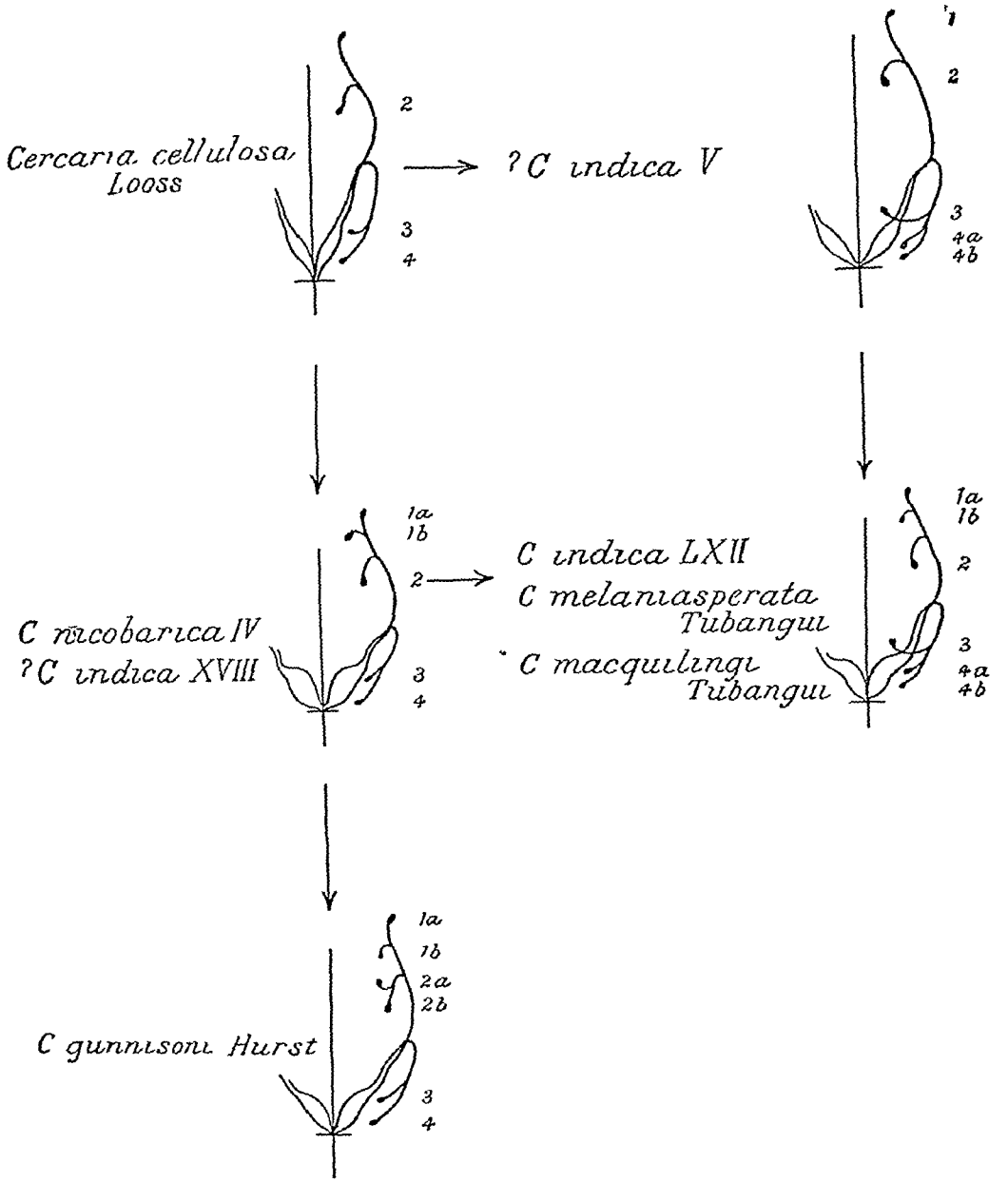
TABLE II

	Cercaria pusilla Looss mm	*Cercaria nicobarica* IV mm
Body-length	0 12	0·092—0·169
Body breadth	0·06	0 040—0·077
Length of tail	0·12	0 084—0·154
Diameter of oral sucker	0·03	0·034
Diameter of acetabulum	0·017	0·015—0·018
Length of stylet	0 019	0·018
Diameter of pharynx	0·011	0 011
Length of sporocyst	0·15	0·132—0·219

In my original account of the 'Pusilla' group (*vide* Sewell, 1922, pp 180-181), I included the following species —

Cercaria pusilla Looss,
Cercaria exigua Looss,
Cercaria indica XVIII Sewell,
Cercaria indica XIX Sewell,
Cercaria indica XL Sewell, and
Cercaria indica XLVI Sewell

I also provisionally included the form described by O'Rooke under the name *Cercaria kansiensis* Since the publication of my paper Tubangui (1928, p 43, Plate III, figs 1–3) has described a further species that clearly must be included here, namely *Cercaria melanaspergata*, which he obtained in the Philippine Islands A study of the excretory systems of these forms indicates that this group is not a homogeneous one but must be subdivided I originally gave the formula of the excretory system as being in all probability $2\times6\times1=12$ flame-cells and of these three are connected with the anterior collecting tube and three with the posterior on each side of the body The formula can thus be written $(1a+1b+2)+(3+4a+4b)$, indicating that the arrangement has been derived from the type of system present in the 'Cellulosa' group, in which there are only $2\times4\times1=8$ flame-cells and which can be graphically represented by the formula $(1+2)+(3+4)$, by the division of flame-cells 1 and 4 respectively into two daughter-cells This type of system has been shown by Tubangui to be present in *Cercaria melanaspergata* and it is also present in *Cercaria indica* LXII Sewell (*vide* Sewell, 1922, addendum, p 1, Text-fig 1) which I regard as a possible connecting link between the 'Pusilla' group and the 'Vingula' group In the present form the excretory system, however, possesses only five pairs of flame-cells and not six, and it seems probable that the same arrangement is present in *Cercaria indica* XVIII Sewell, in which, although I was unable to detect the flame-cells, the anterior collecting tube divides into three branches and the posterior into two only (*cf* Sewell, 1922, Plate XVIII, fig 3) A five pair flame-cell system appears to be present in *Cercaria indica* V Sewell of the 'Parapusilla' group (*vide* Sewell, 1922, p 192, Plate XIX, fig 5) Another stage in the evolution of the Xiphidiocercariæ is represented by *Cercaria gunnisoni* Hurst (1923) This species also possesses a flame-cell system composed of six pairs of flame-cells, but instead of these cells being so arranged that three open into both the anterior and posterior collecting tube, Hurst clearly shows that there are four flame-cells opening into the anterior collecting tube and only two into the posterior tube This arrangement can be graphically represented by the formula $(1a+1b+2a+2b)+(3+4)$ In the Text-figure below I have shown the manner in which these various types of excretory system appear to have evolved from each other



Text-figure.—Showing the manner in which the various excretory systems in certain members of the Xiphidiocercariæ may have evolved from each other

THE 'FURCOCERCOUS' GROUP

Cercaria nicobarica V, sp nov

(Plate XLVII, fig 8)

This cercaria is very active and is a good swimmer, like many other furcocercous cercariæ it moves tail first when swimming, dragging the body

behind It is not able to move with any very great rapidity when crawling by means of its suckers

The body in a moderately extended condition has a length of 0 178 mm and a maximum breadth of 0 096 mm The body is pyriform in outline, the greatest diameter being situated at or a little behind the level of the acetabulum The tail is attached at the posterior end and consists of a tail-stem and a pair of long paddle-shaped furcal rami The tail-stem measures 0 233 mm in length by 0 055 mm in breadth, the furcal rami are 0 219 mm in length

The anterior end of the body is bluntly rounded and is occupied by an organ that is oval or slightly hour-glass shaped according to the degree of contraction or extension of the body, in the contracted condition this organ becomes oval with the long axis transverse, and measures 0 037 mm in length by 0 043 mm transversely This organ appears to partake more of the nature of a penetrating organ than of a definite sucker its structure is not clearly differentiated and it is composed of rounded parenchymatous cells possibly this is due to immaturity I could detect no penetrating spines at the anterior end of the snout, but the anterior two-thirds of the body is armed with minute, closely-set backwardly-directed spines The acetabulum is situated about one-fourth of the body-length from the posterior end, it has a diameter of 0 037 mm and its cavity possesses an H-shaped aperture The tail-stem is not provided with spines Beneath the cuticle there is a layer of rounded cells and internal to this the bulk of the tail is composed of a loose parenchyma of oval cells with granular nuclei, longitudinal striæ denoting the presence of muscle fibres can be made out The furcal rami are not constricted off from the tail-stem and are armed along their edges and on the flat surfaces with closely-set, backwardly-directed spines

The mouth is terminal and leads back through the anterior penetrating(?) organ to a short pre-pharynx that is followed by a well-developed rounded pharynx, that has a diameter of 0 021 mm This is succeeded by a wide œsophagus which passes backwards to the level of the acetabulum and there bifurcates into two short wide cæca that reach back only to the level of the middle of the acetabulum, in some instances, however, this division into two cæca was not shown and the œsophagus terminated in a dilated cavity like a stomach

I was unable to detect any definite salivary gland, I was also unable to trace any salivary ducts My inability to detect these structures was due to the presence beneath the cuticle of numerous small round cells containing short rod-like granules, probably these are cystogenous cells

The excretory bladder is small and circular and is provided with longitudinal muscular fibres Anteriorly the two main collecting tubes open together by a common orifice The terminal part of each main tube is widely dilated and contains within its cavity a number of simple or composite refractile granules This dilated part of the duct appears to be more or less convoluted and is

continued forwards as a narrow tube, in which are situated three ciliated areas, the cilia working backwards towards the bladder

There are five flame-cells in the body in the positions shown in Plate XLVII, fig 8, and a single pair of larger flame-cells is situated at the base of the tail I was unable to trace the greater part of the capillary system but there is a single capillary that runs forwards to the level of the pharynx and receives the capillaries from the two anterior flame-cells in that region A wide caudal excretory canal passes down the tail-stem, but I was unable to detect any 'islet' aperture such as is commonly present in furcocercous cercariæ At the posterior end of the tail the excretory canal bifurcates and a branch passes into each furcal ramus, but I was unable to detect any excretory aperture

The genital organs are represented by a mass of oval or rounded cells lying between the dilated parts of the main excretory ducts and the posterior margin of the acetabulum

The mollusc host is *Melanoides creber* (Reeve) and the locality from which infected snails were obtained was from a small stream near Ray Point, Camorta Island, in the central group of the Nicobar Islands

Development occurs in elongate sausage-shaped sporocysts, one end of which is tapering and is solid The liver of an infected snail has an unhealthy grey colour, occurring in streaks and patches The cercariæ appear to leave the sporocyst while still immature for the substance of the liver was crowded with numerous cercariæ many of which were still immature

According to the most recent attempt at classification, the furcocercous cercariæ are grouped, according to the presence or absence of a definite pharynx, into pharyngeal and apharyngeal series and these are again divided into brevifurcate and longifurcate groups The present form seems to belong to the pharyngeal longifurcate distomes and as such might be considered to be a member of the Holostome series It, however, exhibits certain characters that sharply differentiate it off from the true members of this series

In attempting to classify the various species, the most important system, as has frequently been pointed out, is the excretory system In the possession of five pairs of flame-cells in the body and one pair in the tail-stem the present form agrees with *Cercaria indica* I Sewell (*vide* Sewell, 1922, p 268, Plate XXIX, figs 1 and 2) but in other characters this system differs widely *Cercaria indica* I is the type of the 'Pahila' group and the characteristic features of this group are —

(1) The absence of eyes,
(2) the anterior organ is protrusible but can form a feeble sucker,
(3) the furcal rami are long and are armed with spines along their lateral margins,
(4) a well-developed pharynx is present followed by a long œsophagus and short cæca,
(5) the genital organ is represented by a mass of small cell situated between the excretory bladder and the acetabulum, and

(6) development occurs in elongated thread-like sporocysts

In all these respects the present form agrees but in the excretory system there are several unusual features The excretory bladder is small and compact but the proximal parts of the main excretory tubes are greatly dilated and contain a number of round refractile granules, very similar in appearance to those found in the excretory systems of the Amphistomes and Echinostomes So far as I know, the only other furcocercous cercariæ that have hitherto been recorded as exhibiting such a feature are the members of the 'Vivax' group, in which these granules are quite small (*vide* Sewell, 1922, p 280, Plate XXXI, fig 3), *Cercaria discursata* Ssmitzin and *Cercaria dichotoma* (Muller) *Cercaria discursata*, to judge from the account given by Ssmitzin (1911, p 21, Plate III, figs 45–48), possesses several features in common with the present species At the anterior end there appears to be a rounded sucker, Ssmitzin does not mention this organ in the text but he figures it as present, behind this lies a definite pharynx, followed by an œsophagus and short intestinal cæca, that reach only to the level of the anterior margin of the acetabulum Salivary glands are very much reduced and are represented by two small cells on each side of the body with their respective ducts The excretory bladder is V-shaped and connects with a smaller second bladder that lies at the base of the tail and from this a caudal canal passes down the tail-stem and after bifurcation a branch opens at the tip of each furcal ramus, the excretory bladder is filled with concretions

Cercaria dichotoma Muller has never been adequately described and the accounts given of it differ in various particulars All authors agree, however, in the presence of two suckers, a forked tail with long rami, a wide œsophagus with short intestinal cæca and a U- or lyre-shaped excretory bladder, containing refractile granules Villot (1879) in his account states that he could not detect any pharynx but Lebour (1908, 1912) describes and figures a well-formed one Pilseneer (1906) has also given an account of *Cercaria dichotoma* that agrees for the most part with the descriptions of the other two authors The chief distinction, so far as our present knowledge goes, between the above two species, viz, *Cercaria discursata* Ssmitzin and *C dichotoma* Muller, and the present form lies in the structure of the anterior organ, which in these two species is a definite sucker, whereas in the present species it appears to partake more of the nature of a penetrating organ, though from the undifferentiated character of the cells one cannot be certain on this point, it may be that this apparent difference is due to immaturity in my examples

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Explanation of Plate XLVI

Fig 1 *Cercaria nicobarica* I from the dorsal side

„ 2 *Cercaria nicobarica* II from the ventral aspect

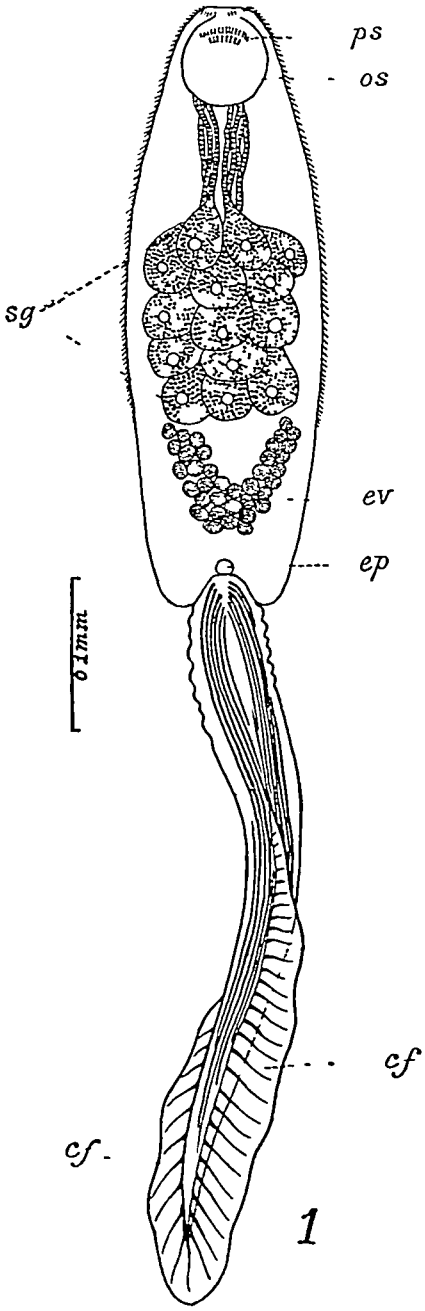
Figs 3*a* and 3*b* Dorsal and Lateral views of the stylet of *Cercaria nicobarica* II

Fig 4 Another example of (?) *Cercaria nicobarica* II from the ventral aspect

List of abbreviations used in Plates XLVI and XLVII

act	anterior collecting tubule
ap	anterior penetrating organ
bi	cerebral ganglion
cet	caudal excretory tube
cf	caudal fin-fold
d	salivary ducts
eg	excretory granules
ep	excretory pore
es	œsophagus
ev	excretory vesicle
fc	flame-cell
g	genital pore
glc	cystogenous cell
ic	intestinal cæcum
m	mouth
os	oral sucker
ov	genital organ
pct	posterior collecting tubule
ph	pharynx
plp	posterior locomotor process
ps	penetrating spines
rd	stomach
sg	salivary gland cell
st	stylet
u	*uterus*
vs	acetabulum

PLATE XLVI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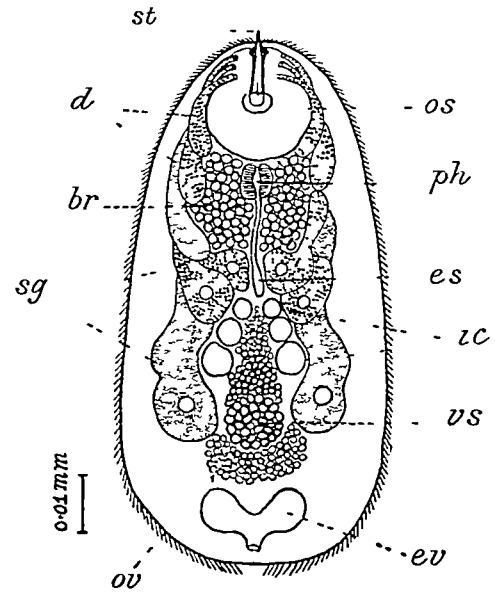
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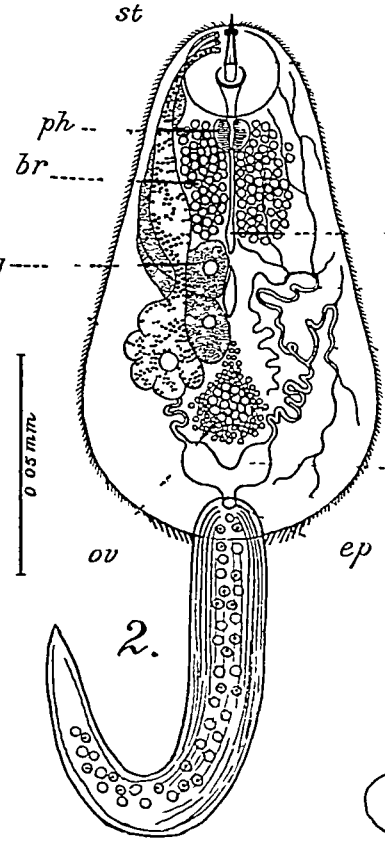
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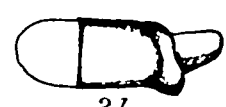
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EXPLANATION

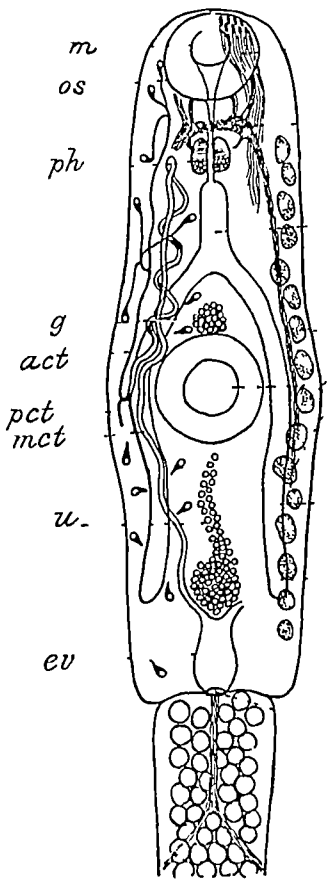
Fig 5 *Cercaria nicobarica* II

„ 6 Redia of *Cercaria nic*

„ 7 *Cercaria nicobarica* IV

„ 8 *Cercaria nicobarica* V

PLATE XLVII



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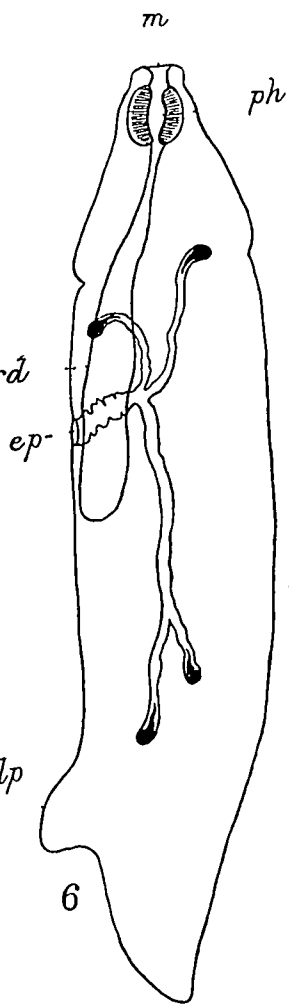

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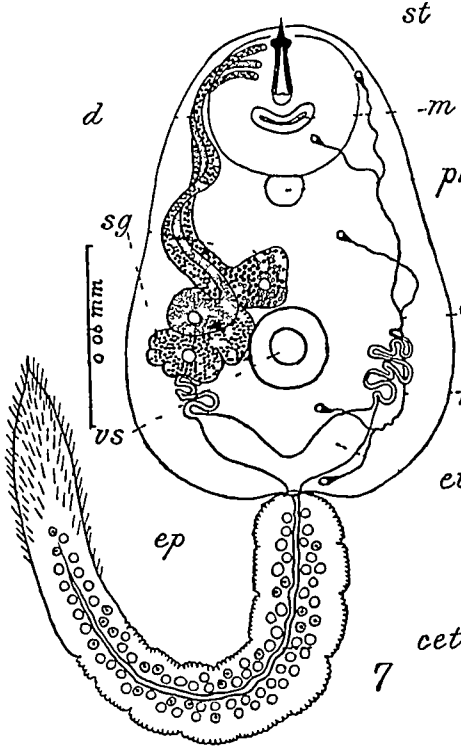

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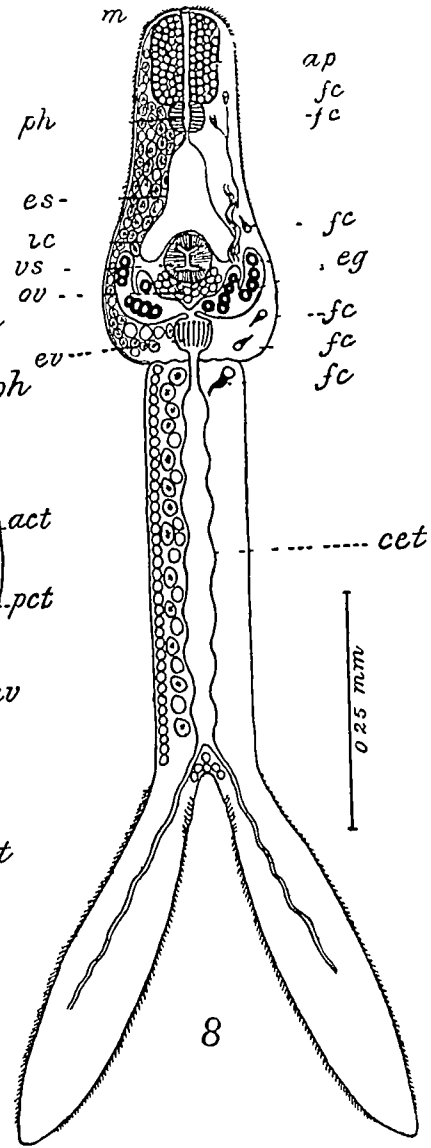

8

THE BASAL METABOLISM OF INDIANS (BENGALIS)

BY

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DURING recent years much interest has been aroused in climatic variations in basal metabolism, but very little work has been done on the basal metabolism of Indians

Eijkmann (1921) could find no significant change in the basal metabolism of Malays when compared to that of Europeans On the contrary De Almeida (1924) obtained results showing marked lowering of basal metabolism in the people of Brazil both of European and African extraction 'De Almeida has developed an interesting theory to account for his findings He believes that in sufficient time all the factors which modify the total metabolism will finally alter the value of the basal metabolism The basal metabolism therefore depends on all the factors which in passing have modified the intensity of the habitual metabolism Among these are muscular work, the level of food intake and the difference between the temperature of the body and the temperature of the air multiplied by the surface area In the tropics all these are lower than in the temperate zone This theory is quite fascinating It would account for the increased metabolism of athletes on account of previous muscular work and of children on account of activity and large food intake It would explain the low metabolism of under-nutrition' (Dubois, 1927)

Montoro (1921-22) working in Havana obtained results very similar to those of De Almeida Earle (1922) stated that the basal metabolic rate of the Chinese was set at a lower pace than that of Western races Yano (1920) observed the low energy metabolism of the Japanese infant and suggested that it might be a characteristic of the Japanese race Takahira (1925), however, after a thorough investigation of Japanese subjects concluded that the basal metabolism of the Japanese showed no marked difference from that of Western races

Knipping (1923) found some lowering of the heat production in Europeans, Malays and Chinese who had lived for some time in the tropics Fleming (1925) in making observations on Filipinos obtained evidence of some lowering of the basal metabolism

Blunt and Dye (1921) studied the metabolism of a Japanese woman living under American conditions. Then subject had a basal metabolism about 14 per cent below the Aub and Dubois prediction.

Macleod Crofts and Benedict (1925) working on Chinese and Japanese women students in America found the average basal metabolism to be about 10 per cent below the Harris and Benedict standards.

Mukherjee (1926) working in Calcutta reported some observations on the basal metabolism of Bengali medical students. The average basal metabolism of his fifteen cases was about —9 per cent, according to the Sanborn standards (i.e., about 14 per cent below the Aub and Dubois prediction). Sokhey (1929) working in Bombay obtained similar results.

Okada, Sakurai and Kameda (1926), however, could find no significant change in basal metabolism of Japanese when compared to that of Western races. Turner (1926) working on a mixed group of Near Eastern subjects found that Armenians gave average metabolism values very close to Aub and Dubois standards but other Near Eastern races gave lower values.

Hembecker (1928) found the metabolism of Eskimos to average 33 per cent above the normal standards. Wardlaw and Horsley (1928) studied the metabolism of Australian aborigines and found them to give low values. Hindmarsh (1927) reported in a study of white students (men and women) living in Sydney, Australia, that the average basal metabolism was markedly low. Turner and Aboushadid (1930) studied the basal metabolism of Syrian women and found that the Aub and Dubois tables give a value at least 12 per cent too high for Syrian women. Tilt (1930) working on Young College women in Florida found the average basal metabolism to be 10 6 per cent (Aub and Dubois standards).

Most of the studies have been made in tropical and sub-tropical countries and the findings tend to indicate a lowered basal metabolism in the people of these places. In discussing this subject Dubois (1927) concludes that the Chinese and the Japanese show a slightly lower metabolism than Americans or Europeans and when these latter come to the tropics they exhibit a gradual decrease in heat production. He is also of opinion that there may be a rather large decrease in heat production in the inhabitants of some tropical countries.

Experimental

The present work is in continuation of the work previously reported by one of us (H. N. M.). Eighteen normal healthy Bengali young men between the ages of 20 to 29 years were studied in the present series. The measurements were made in the post-absorptive condition usually from 14 to 18 hours after the evening meal, with the subject lying down in a state of complete repose. The subject came to the laboratory at about 11 A.M. A preliminary rest period of half to three-quarters of an hour with the subject lying in bed was allowed before the test was started. As the subjects were mostly medical students or

passed graduates of the Carmichael Medical College and as they were made familiar with the working of the apparatus their co-operation was assured

The determination was done with the Douglas bag and the Haldane gas analysis apparatus The usual precautions against leaks were taken and repeated outdoor air analysis were made for checking the gas analysis The experiments were made in autumn, winter and spring

Blood-pressure records were taken with the subject lying in bed a short time after completing the metabolism test

The results are given below in tabular form —

TABLE

Subject number	Age	Height in cm	Weight in kg	Surface area in sq m	Pulse/Respiration	Blood-pressure	Vital capacity in litres	Respiratory quotient	O_2 consumption per minute in c c	Heat production per sq m per hour in calories	Basal metabolism compared to Aub and Dubois standards, per cent
1	26	175 5	59·5	1 72	64/17	110/84	3·40	0 76	164	27·19	—31 2
2	20	163 0	40·5	1 38	80/18			0·97	152	33 10	—16 2
3	27	175·0	63 0	1 75	68/17			0·95	187	31 96	—19·1
4	25	170·0	58·0	1 66	68/18	112/84	4·23	0·94	174	31 28	—20 8
5	23	166 0	44 5	1 46	60/12			0·75	196	38·17	— 3·4
6	22	167 5	57 5	1 64	58/16	105/72	3 38	0·99	211	38·86	— 1 6
7	20	170·0	45 5	1 50	76/20	110/74	3 05	0·97	189	37·88	— 4 1
8	20	167 5	49·5	1 54	72/16	110/84	3·70	0 73	167	30 67	—22·4
9	26	171 3	51 2	1 59	62/13			0·86	213	39·18	— 0 8
10	27	172 5	48·5	1 56	68/17	104/75	3·15	0·75	194	35·36	—10·5
11	26	167·5	47 3	1 51	54/11			0·78	144	27 33	—30·8
12	27	152·5	39·2	1 30	60/15	112/78	3 10	0·79	158	34 92	—11 6
13	26	156·3	49 0	1 46	61/14	102/78	3·40	0·71	150	28·91	—26 8
14	29	177·5	68·0	1 84	74/16	112/75	4·17	0·72	235	36·03	— 8·8
15	24	164 0	47·4	1 49	84/12			0 84	202	39 40	— 0·3
16	21	168·5	52·8	1 59	68/12	110/80	4 31	0·88	194	35 87	— 9 2
17	28	165·0	61 5	1 67	56/10	100/76	3·22	0·84	187	32 59	—17 5
18	26	174 0	59·6	1 71	64/16	112/76	3·68	0·94	224	39 1	— 1·0
Average	25	168·0	52 4	1 58	67/15	108/78	3·56	0·84	186	34 26	—13·3

Discussion

The average basal metabolism of 18 normal Bengali young men studied in the present series is —13·3 per cent (Aub and Dubois standards). Very similar results were obtained and briefly reported by the senior worker (H. N. M.) some years ago. Unfortunately the results were then calculated according to the Sanborn standards (Beaumont and Dodds, 1924).

In a personal communication to one of us (H. N. M.) Miss E. D. Mason working in Madras has informed us that extensive experiments on South Indian women show an even greater deviation (about —18 per cent) from Western standards than does the work on Bengali young men.

Vital capacity.—The vital capacity of 12 of our subjects was measured and the average value is 3·56 litres. The average surface area of these subjects is 1·60 sq. m. According to West (1920) consistent relationship exists between the vital capacity and the area of the body surface,—the actual average for men as found by him being 2·61 litres per sq. m. of body surface. In our cases the average is 2·225 litres per sq. m. of body surface (a deviation of about —14·8 per cent from normal Western standards). Foster and Hsieh (1923) obtained similar low values in Chinese students.

According to the report on the Students' Welfare Scheme of the University of Calcutta (1928) the average vital capacity of Bengali students (men) appears to be even lower than our average.

Blood-pressure.—The average systolic blood-pressure of the 12 subjects is 108 and the diastolic pressure is 78. The average pulse pressure is 30 and appears to be low compared to Western standards.

It is perhaps not out of place here to mention the variations from normal Western standards of the various blood constituents in Bengalis. The low hæmoglobin contents of the blood of Bengalis was observed by McCay (1912).

Mukherjee (1923, 1925*a*, 1925*b*, and 1928) estimated the non-protein nitrogen, urea N, uric acid, cholesterol contents, etc., of the blood of young Bengalis. He found the N. P. N. and urea N. contents to be low but the uric acid was found to be high. The cholesterol content was found to be definitely low (and perhaps even the calcium content is slightly low).

Mukherjee also observed the markedly high sedimentation velocity of erythrocytes in Bengalis.

Boyd and Roy (1928) also observed the low cholesterol content of the blood of Bengalis.

McCay (1912) observed the low urinary nitrogen of Bengalis.

While these observations may or may not have any relation with basal metabolism it is not possible to overlook them and it suggests to oneself that the low basal metabolism of Bengalis is perhaps due to two factors (1) climatic and (2) nutritional.

The low nitrogen content of the Bengali diet is well known and the diet is also poor in fats. McCarrison (1929) studied the relative biological values

of the different Indian diets, and found the Bengali diet to be poor in suitable proteins, vitamins and mineral elements

Summary

Basal metabolism measurements are reported on 18 normal Bengali young men The findings are summarized below —

(1) The basal metabolism of Bengali young men averages —13 3 per cent (Aub and Dubois standards)

(2) The vital capacity is markedly low, i e , on the average about 14 8 per cent lower than Western standards

(3) The pulse pressure appears to be low

(4) The low basal metabolism of Bengalis is perhaps due to climatic and nutritional causes

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A NOTE ON BLOOD CHANGES IN FILARIASIS

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INTRODUCTION

THE observations were undertaken during the course of my employment under the Indian Research Fund Association, Simla, and while serving under the Helminthological Inquiry, Bihar and Orissa The period of observations lasted from October 1929 to March 1930

MATERIAL AND TECHNIQUE

The material for observation was collected from cases investigated at the towns of Gaya, Monghyr and Kharagpur (Monghyr district)

The blood material was taken in quantities of 20 c mm at different hours of the day and night The blood films were treated by recognized methods for staining The microfilariæ found in the peripheral blood were identified as *F bancrofti*, and the cases classed as positive In many cases stools were examined for the presence of helminthic ova The counts of the red and white cells were made by Thoma Zeiss's Hæmocytometer The hæmoglobin percentage was arrived at by Gower's method Differential leucocytic counts were made after staining the films by Giemsa stain

RESULTS OBTAINED

The results are incorporated under Tables I and II Table I shows blood changes in cases in which the embryos of *F bancrofti* were found in the peripheral blood, and Table II shows similar changes in the absence of microfilariæ in the peripheral blood

The tables are explanatory in themselves

TABLE I

Showing blood changes in filariasis (F bancrofti)

A Cases showing microfilariæ in the peripheral blood

Details of the case sex, age, total parasites per 20 c.mm., duration signs, helminth ova	Hb per cent	R B C per c.mm. (first figures millions)	W B C per c.mm. Examination same hours		Differential leucocyte count Examination same hours									
					Day					Night				
			Day	Night	P	E	L	T	H	P	E	L	T	H
M, 29 yrs par 25, 2 yrs, orchitis, ova nil	80	5,132	7,500	7,500	57	6	30	3	4	58	7	28	4	3
M, 40 yrs, par 1, 4 yrs, hydrocele, *A lumbricoides*	70	5,240	6,250	6,562	53	8	31	3	2	52	8	33	4	3
M, 15 yrs, par 10, data, negative	68	4,896	7,500	7,187	57	5	35	1	2	59	5	32	2	2
M, 32 yrs, par 40, data, negative	65	4,784	5,625	5,937	59	4	33	1	3	60	4	32	2	2
M, 22 yrs, par 388, 2 yrs, orchitis, ova, nil	62	4,608	5,312	5,312	54	10	31	1	4	55	10	30	2	3
M, 31 yrs, par 8, 8 yrs, elephantoid scrotum, ova, nil	82	5,452	6,500		64	4	26	2	4					
M, 35 yrs, par 3, 2 months, hydrocele, ova, nil	84	6,416	7,000		62	5	26	3	4					
M, 30 yrs, par 8, data, negative	76	6,080	7,360		64	6	25	2	3					
M, 20 yrs, par 6, data, negative	80	5,640	7,400		62	6	28	1	3					
M, 55 yrs, par 16, data, negative	80	6,144	8,750		66	6	24	2	2					
M, 30 yrs, par 12, data, negative	75	5,648	6,875		61	5	29	3	2					
M, 34 yrs, par 66, data, negative	72	5,384	5,937		58	5	32	2	3					
F, 18 yrs, par 6, 3 months, fever, ova, nil	70	5,008	6,875		56	6	33	2	3					
Average Parasites 45	74	5,440	6,860	6,499	59	6	30	2	3	57	7	31	3	2

TABLE II

Showing blood changes in filariasis (F bancrofti)

B Cases not showing microfilariæ in the peripheral blood

Details of the case sex, age, duration, signs helminth ova	Hb per cent	R B C per c mm (First in figures millions)	W B C PER C MM EXAMINATION SAME HOURS		DIFFERENTIAL LEUCOCYTE COUNT EXAMINATION SAME HOURS									
					Day					Night				
			Day	Night	P	E	L	T	H	P	E	L	T	H
M 43 yrs 6 yrs, elephantoid scrotum, ova nil	65	5,504	7,500	6,875	60	4	30	2	4	62	4	28	3	3
M, 52 yrs 1 yr, elephantoid scrotum, ova nil	65	5,280	6,562	7,187	56	7	28	4	5	58	6	28	4	4
M 20 yrs, 3½ yrs, hydrocele, ova nil	64	4,896	5,312	5,625	52	8	34	3	3	54	8	32	3	3
M, 61 yrs 18 yrs, hydrocele ova nil	68	4,824	5,937	5,625	52	6	36	3	3	53	6	35	4	2
M 40 yrs 1 yr, elephantoid leg, ova, nil	62	4,760	6,562	6,875	54	7	34	3	2	55	7	32	3	3
M 25 yrs 2 yrs, elephantoid leg, ova, *A duodenale*	80	5,640	7,500		60	3	30	3	4					
M 45 yrs, 3 yrs, elephantoid leg, ova, nil	70	4,768	6,200		62	6	25	4	3					
M 60 yrs 4 months, hydrocele	64	4,152	6,200		64	4	28	1	3					
M 29 yrs 4 yrs, orchitis, ova, nil	75	5,936	8,000		59	5	29	3	4					
M 25 yrs 1 yr, inguinal adenitis	80	5,672	7,500		64	4	25	3	4					
M 36 yrs, 10 yrs, hydrocele ova, *A lumbricoides*	70	5,728	8,125		62	4	27	4	3					
M 30 yrs, 2 yrs, orchitis, ova, nil	65	5,408	6,250		58	5	30	3	4					
M 20 yrs, 1 yr, elephantoid leg ova, nil	64	5,088	6,875		52	12	30	2	4					
M 24 yrs, 3 yrs, hydrocele ova, nil	68	5,368	7,187		54	8	32	1	5					
M 20 yrs, 8 months orchitis ova, nil	60	4,952	5,625		56	6	31	2	5					
Average .	68	5,198	6,756	6,437	57	6	30	3	4	56	6	31	4	3

SUMMARY OF RESULTS

(1) The variation in the percentage of hæmoglobin is between 62 and 84, average 74, in the positive cases, and 62 and 80, average 68, in the negative cases

The normal mean hæmoglobin value in the adults of Gaya district varies between 72 and 75 per cent (Korke, 1927) It appears therefore that there is no marked fall in the hæmoglobin percentage from the standard

(2) The mean value of the red blood cells per c mm is normal in the positive (5,440,000) and negative (5,198,000) cases of filariasis

(3) Similarly the mean value of the white blood cells per c mm (day and night counts) is nearly normal (positive cases, average, 6,743, negative cases, average, 6,675)

(4) There appears to be no appreciable difference between the *differential leucocytic count* made during the identical hours of day and night, except for the fact that the eosinophile cells show a tendency towards a rise and polymorphonuclear cells towards a fall, both in the positive and negative cases of filariasis This change appears to be due to filariasis, as the presence of helminthic ova found in the cases appears to be negligible

(5) The number of microfilariæ in the peripheral blood does not seem to influence the normal value of the red cells, but there is a suggestion in the case of white cells which are below normal (*vide* cases 4, 5 and 12, Table I)

REFERENCE

KORKE, V T (1930) *Transactions, F E A T M 7th Congress, (Calcutta,* 1927), III, p 258

NOTES ON SOME INDIAN SPECIES OF THE GENUS *PHLEBOTOMUS*

Part XXVI.

PHLEBOTOMUS ELEANORÆ N SP

BY

MAJOR J A SINTON, I M S

(*Malaria Survey of India, Kasauli*)

[Received for publication, August 12, 1930]

AMONG a collection of sandflies made on 3rd April, 1930, in human habitations on the Imperial Cattle Farm, Karnal, Punjab, was found one male of a new species It is proposed that this insect be named *Phlebotomus eleanoræ*

Phlebotomus eleanoræ N SP (♂)

The species belongs to the erect-haired group The specimen was contained in a collection consisting of *P minutus, P babu, P papatasi* and *P sergenti* and was not differentiated from the latter species prior to mounting For this reason no description can be given of its appearance in the fresh state

Appearance of stained and mounted specimen

The measurements of the specimen and the relative lengths of its different appendages are given in the attached table

The insect is a comparatively large species, measuring about 2 9 mms in total length

The *Pharynx* (Plate XLVIII, fig 5) is about 3 9 times as long as wide and its broadest part is only about 1 6 times its narrowest width The pharyngeal armature consists of a series of faint transverse curved ridges, some of which have irregularly notched posterior margins The buccal armature is poorly developed and the pigmented area is absent

The *Antennæ* (Plate XLVIII, figs 3 and 4) have paired geniculate spines on all segments from III to XV These are short and stout and do not extend

as far as the succeeding inter-segmental articulation Segment III is shorter than the combined lengths of segments IV and V, but more than half the length of segments XII to XVI

The *Palps* (Plate XLVIII, fig 2) have a formula of 1, 2, 4, 3 5, the relative lengths of the different segments being 3 4, 8 8, 12 2, 10, 22 4 The combined length of segments 1 and 2 equal that of 3, which is distinctly incrassate Newstead's spines number 15–18 and are situated at the junction of the middle and basal thirds of the 3rd segment

The *Wing* (Plate XLVIII fig 1) is about 3½ times as long as broad The distance *c* is very small and the subcostal vein ends slightly proximal to the commencement of the 3rd vein

The *Hind Leg* is relatively long and measures slightly more than the body length The femur forms one-fourth of the leg and is approximately of the same length as tarsal segments 2–5 The tibia is about one-third the length of the leg

The *Male Genitalia* (Plate XLVIII figs 6, 7, 8 and 9) have been mounted so as to give a dorso-ventral view, and are characteristic in shape The proximal segment of the *superior clasper* is broad and has a small tubercle arising from its internal surface near the base This tubercle carries about 10 hairs which are about 90 μ long and have curved tips The tubercle closely resemble that seen in *P sergenti* Parrot This segment is about equal in length to the intermediate appendage but is distinctly shorter than the inferior clasper The proximal portion of the distal segment of this clasper is stout and irregular in shape, while the distal half is much narrower and cylindrical It bears 5 stout curved spines each about 0 1 mm long These have the following arrangement an apical pair arising very close together, a single spine arising from a stout tubercle on the inner side of the middle of the segment, and two spines arising from smaller tubercles situated on the inner and outer sides of the segment at the junction of its basal and outer thirds Of the last spines the outer is more slender than the others

The *intromittent organ* shows rounded and slightly dilated tips, resembling those in *P sergenti* The *genital filament* is not protruded The *pompetta* lies across the junction of abdominal segments 7 and 8 with its major portion in the former

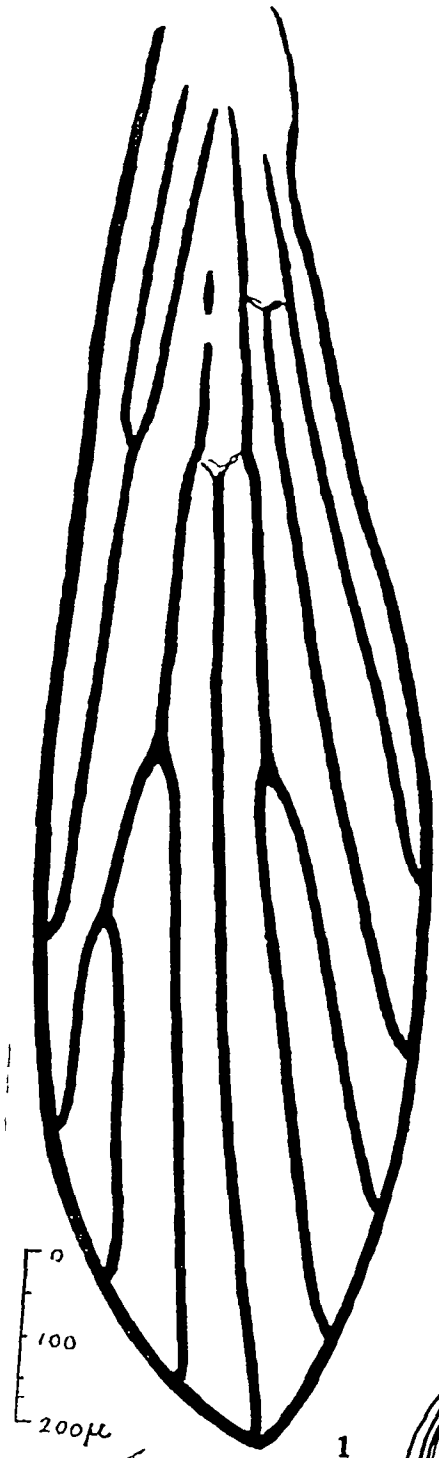
Diagnostic characters

The shape of the distal segment and the distribution of its spines differentiate this species from all the other erect-haired species The presence of a tubercle on the inner surface of the basal segment of the superior clasper has only been described in *P papatasii, P caucasicus* (*P h* Popoff) and *P sergenti* In *P papatasii* this structure is very small, in *P caucasicus* it is large, while in *P sergenti* it is similar to that seen in *P eleanorae*

Phlebotomus eleanoræ n sp (♂)

	Structure	Lengths in mms	Relative lengths, formulæ, etc
Body	Clypeus and head	0·385	
	Thorax	0·670	
	Abdomen, proper	1·600	
	Sup clasper, seg 1	0·255	
	Total length	2·9	= 1·77 × wing length, ≷ hind leg
Mouth	Labium	0·220	$\frac{P}{L} = 3{\cdot}27$ $\frac{P}{E} = 4{\cdot}37$
	Epipharynx	0·160	
	Pharynx, length	0·200	= 3·9 × breadth
	Pharynx, breadth	0·051	
Antenna	Segment III	0·190	III < IV + V IV = V = VI
	Segment IV	0·100	
	Segment V	0·100	IV + V + VI < XII–XVI
	Segment VI	0·100	Antennal formula $\frac{2}{\text{III–XV}}$
	Segments XII–XVI	0·350	
	Total length	1·400	= 7·37 × IIIrd = 4 × XII–XVIth
Palp	Segment 1	0·042	Formula 1 2 4, 3, 5
	Segment 2	0·108	Relative lengths, 3·4, 8·8, 12·2, 10, 22·4
	Segment 3	0·150	
	Segment 4	0·123	1 + 2 = 3
	Segment 5	0·276	
	Total length	0·700	

	Structure	Lengths in mms	Relative lengths, formulæ, etc
Wing	Length	1·613	= .57 × breadth
	Breadth	0·113	
	α	0·257	$\frac{\alpha}{\beta} = 1.28\frac{\beta}{\gamma} = 0.66\frac{\alpha}{\gamma} = 0.86\frac{\gamma}{\alpha} = 0.09$
	β	0·200	
	γ	0·300	
	δ	0·021	$\frac{\alpha}{\epsilon} = 0.62\frac{\theta}{\epsilon} = 1.72\frac{\alpha+\beta}{\theta} = 0.64$
	ϵ	0·111	
	θ	0·711	$\frac{\text{Wing}}{\theta} = 2.30$
	τ	0·070	
Hind leg	Femur	0·757	= ½ length leg, = tarsal segs 2–5
	Tibia	1·028	= ⅓ length leg
	Tarsus, seg 1	0·550	
	Tarsus, segs 2–5	0·743	
	Total length	3·08	(Not including coxa and trochanter)
Genitalia	Sup claspei, seg 1	0·255	= 1·57 × 2nd seg, = intermed app = 0·81 × inf clasper
	Sup claspei, seg 2	0·162	
	Interined appendage	0·258	
	Intromittent organ	0·171	
	Inferior claspei	0·312	= 1·30 × subgen lamellæ
	Subgenital lamellæ	0·240	
	Pompetta	0·120	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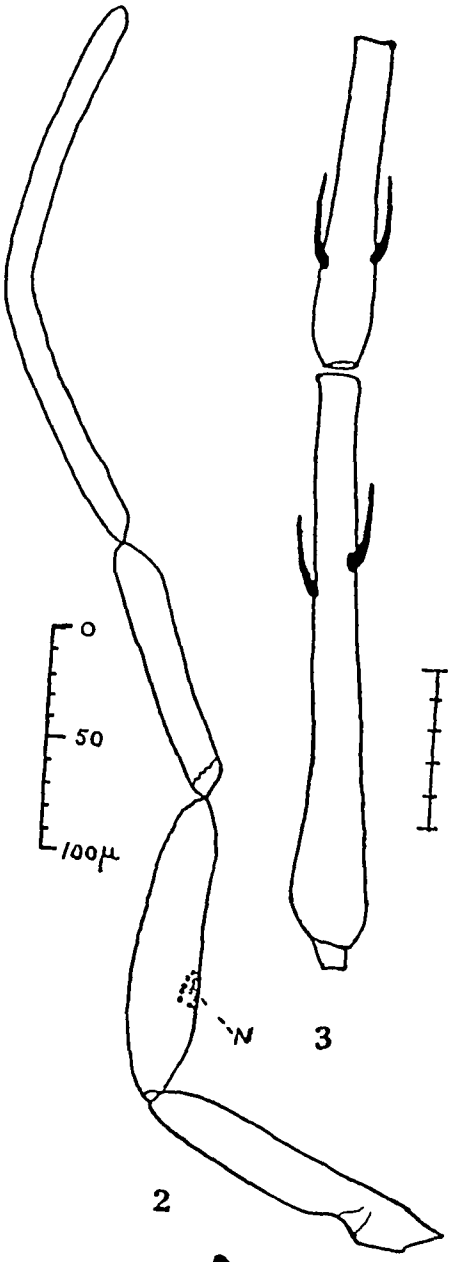
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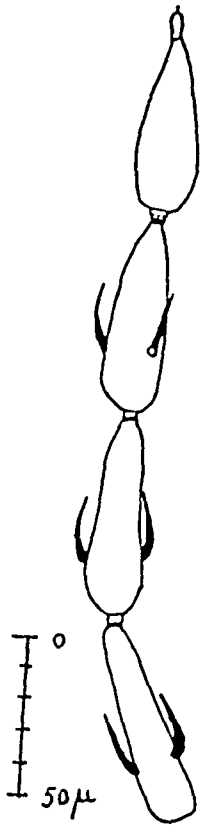
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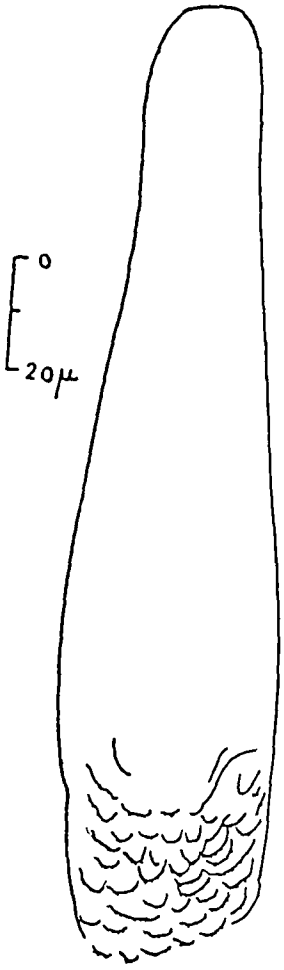
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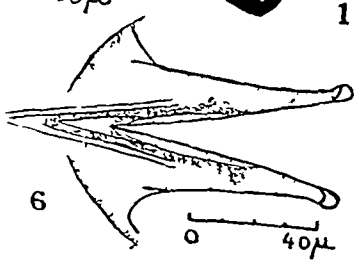
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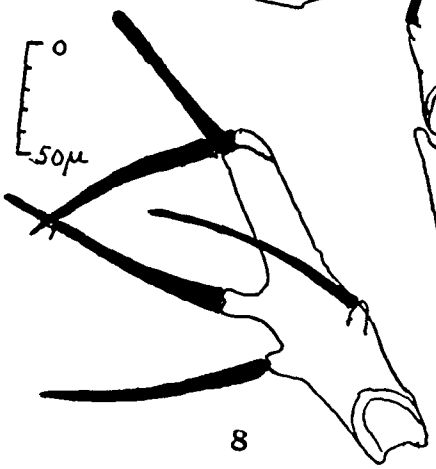
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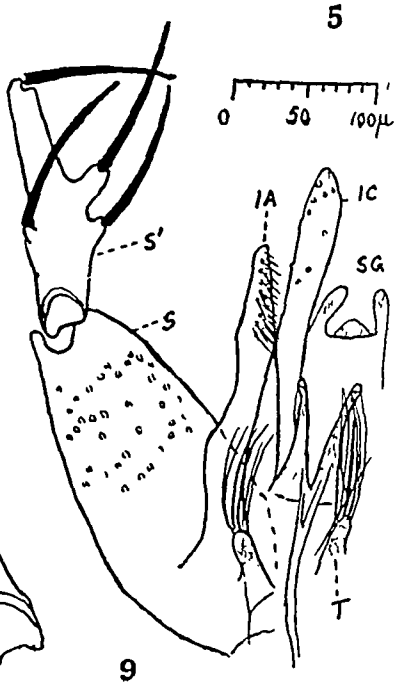
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Explanation of Plate XLVIII

Phlebotomus eleanoræ (♂)

Fig 1 Wing
„ 2 Palp showing position of Newstead's spines (N)
„ 3 Segments III and IV of antenna
„ 4 Segments XIII–XVI of antenna
„ 5 Dorsal view of pharynx
„ 6 Dorsal view of intromittent organ
„ 7 Tubercle on basal segment of superior clasper
„ 8 Distal segment of superior clasper
„ 9 Male genitalia of one side S, basal segment of superior clasper S′, distal segment of superior clasper, with one apical spine missing IA, intermediate appendage IC, inferior clasper SG, subgenital lamellæ T, tubercle on basal segment of superior clasper

NOTES ON SOME INDIAN SPECIES OF THE GENUS *PHLEBOTOMUS*

Part XXVII

PHLEBOTOMUS BAILYI N SP

BY

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(*Malaria Survey of India, Kasauli*)

[Received for publication, August 15, 1930]

As was pointed out in previous papers there were included in the *minutus* group of sandflies in India a number of species which had been diagnosed provisionally as *P minutus* var The older methods of identification indicated that these were probably separate species, while by the newer aids to diagnosis it has been possible to divide this group into at least seven different species, each with well-marked specific characters During the last few years I have collected a large number of specimens of a new species of this group from different parts of India I am also indebted to Major D Clyde, I M S, to Mr R Senior White, F R S E, and to Subedar J D Baily, I M D, for specimens from various localities The last worker especially has supplied me with numerous specimens from the Central Provinces, Sind and the Punjab, and I have great pleasure in suggesting the name *Phlebotomus bailyi* for this species

MATERIAL

More than one hundred specimens of this species have been collected and these have been found in the following localities —

Punjab—Lahore, Karnal, Chandigarh, Ambala District, Kasauli, Sanawar, Gaikhal and Kuthar State, Simla Hills

United Provinces—Saharanpore, Roorkee and Hardwar, Saharanpore District, Dehra Dun, Laharpur, Sitapur District

Bihar and Orissa—Barhi, Hazaribagh, Nauamandi and Ulibura, Singhbhum District, Titlagarh, Patna State

Central Provinces—Nagpur, Kamptee, Pachmarhi, Harda, Itarsi, and Piparia, Hoshangabad District, Dodhei, Sausar, Parasia and Chhindwara, Chhindwara District, Khandwa, Nimar District

Madras—Bissem Kattack, Satikona and Sikar Kopa, Vizagapatam Agency

N.-W. F. Province—Dera Ismail Khan and Landola, Waziristan

Sind—Walid, Larkhana District, Tando Mahomed Khan, Hyderabad District, Panoakil, Sukkur District

From this list it can be seen that this species exists at altitudes from a few feet above sea-level up to 6,000 feet, and has a very wide distribution in India. It is, however, a much rarer form than *P. babu*, and usually forms not more than 3 per cent. of the *minutus* group in any mixed catch from the plains, while in the hills it is relatively more abundant and is found along with *P. montanus*, *P. major* and *P. chinensis*.

Phlebotomus bailyi n. sp. (♀)

The type specimens described here were all collected during July in a dwelling house in Kasauli, Simla Hills, at a height of about 6,000 feet. These montane specimens show slight but definite differences from specimens collected from the plains, so it is proposed that the latter variety be called, for the sake of convenience, *P. bailyi* var. *campestri*.

The species is a dark medium-sized one, slightly larger than the more common members of the *minutus* group. The very pale pleuræ are in marked contrast to the dark integument of the rest of the body and give the insect the general appearance of a small specimen of *P. argentipes*.

When examined under the microscope it has a very dark greyish brown, almost black, general appearance and some specimens show a lapis-lazuli iridescence of the hairs and scales of the body and its appendages. The integument of the pleuræ, coxæ, trochanters and upper parts of the femora is yellowish, in marked contrast to the very dark brown appearance of the rest of the insect. The abdominal hairs are recumbent on the dorsum, while those of the venter have a ruffled appearance and are inclined to be semi-recumbent. The dorsal hairs are dark greyish brown, those on the venter being slightly paler with a yellowish tinge. The genitalia are usually lighter in colour than the rest of the abdomen. The erect hairs on the dorsum of the thorax and head are greyish brown with darker tips. The wings are covered with dark grey hairs, infuscated towards the alar margins. They have a golden-bluish or lapis-lazuli iridescence. The halteres are almost black. The legs are a very dark grey with a silvery sheen by reflected light. The palps and antennæ are dark grey.

Appearances in stained and mounted specimens

The measurements of the Type and three Co-type females are given in Table I, in which the relative lengths and ratios have also been given from the study of eight specimens.

The average *total length* of these specimens was 2 6 mms (2 4–2 75 mms)

The posterior portion of the *buccal cavity* (Plate XLIX, fig 6) has the appearance of being formed of two pointed ear-like projections, which are more prominent in var *campesteri* (Plate XLIX, fig 7) This is very similar to that seen in *P christophersi* (*cf* Sinton, 1927*a*, Plate IX, figs 10 and 11) The pigmented area is completely absent in the type species but in var *campesteri* there is a small but distinct area, often square or rectangular in shape, with a solid appearance The buccal armature consists of a large number of very small fine teeth, which tend to be arranged in several distinct rows, especially laterally The hypopharynx is stout with a wide base and the ratio palp over epipharynx averages about 4 5

The length of the *pharynx* (Plate XLIX, fig 3) is about 2 8–3 times that of its greatest breadth, while the narrow anterior portion is about twice this breadth The armature consists of a series of transverse curved ridges, the posterior margins of which carry small short teeth

The *antennæ* (Plate XLIX, figs 4 and 5) have a total length about 7 times that of segment III and about 4 3 times that of segments XII–XVI The IIIrd segment is about 200μ long and extends as far as the tip of the proboscis The length of this segment is slightly greater than the combined lengths of segment IV and V, while the lengths of IV, V and VI are about 0 85 times that of segments XII–XVI The antennal formula is 2 over III to XV, and the spines are of medium length and stout in character

The *palps* (Plate XLIX, fig 2) have a formula of *1, 2, 3, 4, 5* and the relative lengths of the segments averaged 2 4, 6 6, 9 0, 10, 21 6 The combined lengths of segments 1 and 2 equal that of 3, while segment 4 forms about 1|5th of the palpal length The ratio palp over labium is about 3 2 Newstead's spines are situated on the basal third of the 3rd segment and number about 40 This segment is markedly incrassate

The *wing* (Plate XLIX, fig 1) is about 4 times as long as broad The venation shows considerable variation as shown in Table I The ratio δ over α is about 0 47

The *hind leg* is about 4 times the length of its femur and 3 times that of its tibia The latter segment is about twice the length of the 1st tarsal segment

The *female genitalia* (Plate XLIX, figs 8 and 9) have a smooth spermatheca, which is nearly twice as long as broad It corresponds to Type B of Adler and Theodor (1927) and has a narrow duct The post-genital ridge carries 3 or 4 spines

Phlebotomus barlyi var *campesteri*, the variety of this species found on the plains, differs from the type in the following points the buccal region has a small angular solid-looking pigmented area (Plate XLIX, fig 7), the length of segment III of the antenna is usually less than 200μ, while in the type it is generally greater than this, the length of this segment in the variety usually equals the combined lengths of segments IV and V, while in the type it is greater, the total length of the antenna is about 7½ times segment III as

compared with only 7 times in the type, the ratio ♂ over ♀ is usually about 0 5, while in the type it is less, the body length of the type species is usually greater than that of the variety, the outline of the posterior portion of the buccal cavity is wider and more basin-shaped than in the type

Differential diagnosis

The marked contrast between the light pleurae and the dark integument of the rest of the body, when present in a medium-sized recumbent-haired species with a comparatively long ♂, usually indicates this species

The absence of erect hairs on the dorsum of abdominal segments 2 to 7 and the smooth outline of the spermathecae differentiates this species from the members of the erect-haired group The shape of the posterior portion of the buccal cavity rather resembles that of *P christophersi*, but the numerous small teeth of the buccal armature in *P bailyi* are very different from the few large ones in the erect-haired species

The poorly developed buccal armature and pigmented area in the females of this species at once distinguish it from the other Indian members of the recumbent-haired group The narrow wing with a relatively long ♀ and ♂ and the long IIIrd antennal segment resembles those seen in *P montanus*, with which it may be associated in the Himalayan foot-hills The well-developed pigmented area and buccal armature in the latter species make the diagnosis easy (*cf* Sinton, 1927, Plate VIII, fig 10)

P bailyi shows certain resemblances to the Philippine species, *P mcmc* Banks, but if the description given here be compared with that of *P mcmc* given by Sinton (1930) the following differences will be noted In the latter species the posterior portion of the buccal cavity is not so expanded, the pigmented area is more rounded and less solid looking, the posterior end of the pharynx is more constricted, the wing is relatively broader, being about 3½ times as long as broad, the IIIrd segment of the antenna is shorter, Newstead's spines are situated on the middle not the basal third of the 3rd palpal segment and are fewer in number

TABLE I

Phlebotomus bailyi n sp (♀)

	Structure	Lengths in mm of specimens number —				Remarks, average relative lengths, etc*
		1	2	3	4	
Body	Clypeus and head	0 400	0·343	0 343	0·370	
	Thorax	0·643	0 570	0·714	0·628	
	Abdomen proper	1 457	1 357	1 530	1 500	
	Sup clasper	0·170	0·143	0·170	0 143	
	Total length	2 67	2·41	2·75	2 64	

*Averages taken from eight specimens

TABLE I—*concld*

	Structure	Lengths in mm of specimens number — 1	2	3	4	Remarks, average relative lengths, etc *
Mouth	Labium	0·234	0 200	0 243	0 221	$\frac{P}{L}=3{\cdot}18$ $\frac{P}{E}=4\,5$
	Epipharynx	0·165	0·140	0·168	0·160	
	Pharynx, length	0 168	0·150	0 174	0·160	$=2{\cdot}8\times$ breadth
	Pharynx, breadth	0 063	0 054	0·058	0 048	
Antenna	Segment III	0 216	0·180	0·216	0·195	$>$IV + V IV = V = VI
	Segment IV	0·096	0·087	0·093	0 090	IV + V + VI $<$ XII–XVI
	Segment V	0 100	0·090	0·096	0 090	Antennal formula $\frac{2}{\text{III–XV}}$
	Segment VI	0 100	0 090	0·102	0·093	
	Segs XII–XVI	0·342	0·310	0·345	0 310	$=1\,6\times$ IIIrd
	Total length	1 500	1 310	1 500	1 360	$=7\times$ IIIrd, $4\,3\times$ XII–XVIth
Palp	Segment 1	0 036	0 033	0·040	0 033	Formula, 1, 2, 3, 4, 5
	Segment 2	0·100	0·087	0 100	0·093	Relative lengths, 2 4, 6 6, 9, 10, $\frac{21\,6}{}$
	Segment 3	0 135	0 120	0·138	0·129	$=1+2$
	Segment 4	0·150	0·132	0·153	0·141	$=1/5$th palpal length
	Segment 5	0·315	0·270	0 339	0·318	
	Total length	0·736	0 642	0·768	0 714	
Wing	Length	2·200	1 630	2 000	1 830	$=4\times$ breadth
	Breadth	0 500	0 400	0·485	0 443	
	α	0·385	0 243	0·393	0·328	$\frac{\alpha}{\beta}=0\,90$ $\frac{\beta}{\gamma}=1\,20$
	β	0 400	0·328	0·393	0·314	
	γ	0·285	0·328	0·300	0·300	$\frac{\alpha}{\gamma}=1\,2$ $\frac{\delta}{\alpha}=0\,47$
	δ	0·200	0·100	0·185	0·143	
	ϵ	0·543	0·370	0·570	0 470	$\frac{\alpha}{\epsilon}=0{\cdot}68$ $\frac{\theta}{\epsilon}=2{\cdot}0$
	θ	1 071	0·814	1 070	0·914	
	τ	0·100	0·128	0·114	0·030	$\frac{\alpha+\beta}{\theta}=0{\cdot}71$ $\frac{\text{Wing}}{\theta}=1\,9$
Hind leg	Femur	0·800	0 657	0·800	0·714	$=\frac{1}{4}$ leg
	Tibia	1 057	0·871	1 057	0·928	$=\frac{1}{3}$ leg, $2\times$ tarsus seg 1
	Tarsus, seg 1	0·543	0 443	0·543	0·485	
	Tarsus, segs 2–5	0·714	0·585	0·700	0·643	
	Total length	3·1	2·55	3 1	2 77	(Not including coxa and trochanter)
	Sperm, length	0·075	0·060	0 072	0·075	$=1\,8\times$ breadth
	Sperm, breadth	0·042	0·033	0·039	0·037	

* Averages taken from eight specimens

Phlebotomus baliyi n sp (♂)

The male resembles the female in general appearance, being of a dark greyish brown colour, but is often slightly lighter in hue The inferior clasper carries a number of silvery scales

Appearances in stained and mounted specimens

The measurements and relative lengths of the different appendages, etc, from four specimens are given in Table II

The *total length* varied from 2 6 to 2 8 mms

The *buccal cavity* (Plate L, figs 5 and 6) shows an armature consisting of a large number of very small fine teeth which tend to be arranged in two or three rows The pigmented area is absent in type specimens but many specimens of var *campestri* have a small but distinct pigmented area The ratio palp over epipharynx averages about 4 9

The length of the *pharynx* (Plate L, fig 9) is about 3 2 times its breadth, while the narrow anterior portion is about twice this breadth The armature resembles that in the female but is slightly less developed

The *antennæ* (Plate L, figs 3 and 4) have a formula of 1 over III to XV and the geniculate spines are comparatively short Segment III is longer than in the female and reaches beyond the tip of the proboscis Its length is approximately equal to that of segments IV and V combined The total length of the antenna is about 6 8 times that of segment III, about 4 6 times that of segments XII–XVI, and 0 9 times the wing length

The *palps* (Plate L, fig 2) have a formula of 1, 2, 3, 4, 5 and the relative lengths of the segments averaged 2 2, 6 1, 8 5, 10, 21 1 Segment 4 is relatively longer than in the female, and forms more than 1|5th of the palp The combined lengths of segments 1 and 2 equal that of 3, which does not show the marked incrassation seen in the female Newstead's spines are about 10 in number and are situated on the distal end of the basal fourth of the 3rd segment The ratio palp over labium averaged about 3 5

The *wing* (Plate L, fig 1) is narrower than in the female being 5 times as long as broad The ratio δ over α is also shorter, being about 0 39

The *hind leg* is longer than the body length and is about half as long again as the wing The femur forms about one-fourth and the tibia about one-third of the leg length

The *male genitalia* (Plate L, figs 7 and 8) are of the *minutus* type but larger than usual The proximal segment of the *superior clasper* is about 2 3 times the length of the distal one and about 1 32 times that of the intermediate appendage The distal segment carries 4 stout curved spines each about 100 μ in length These spines are placed two apically and two very sub-apically, while the segment is distinctly broadened at the origin of the latter spines The small non-deciduous spine is more distal than usual in the *minutus* group, its point of origin being almost level with the sub-apical spines The *inferior*

TABLE II

Phlebotomus bailyi n sp (♂)

	Structure	Lengths in mm of specimens number — 1	2	3	4	Remarks, average relative lengths, etc*
Body	Clypeus and head	0·357	0·343	0·330	0·370	
	Thorax	0·543	0·528	0 514	0·643	
	Abdomen proper	1 530	1 530	1 457	1 500	
	Sup clasper, seg 1	0·276	0 290	0·285	0·300	
	Total length	2 7	2·7	2·6	2·8	
Mouth	Labium	0·195	0 214	0·200	0 225	$\frac{P}{L}=3\,5$ $\frac{P}{E}=4\,9$
	Epipharynx	0 140	0·150		0·172	
	Pharynx, length	0·147	0 156	0·156	0 170	3·2 × breadth
	Pharynx, breadth	0·048	0·048		0·054	
Antenna	Segment III	0·230	0·252	0·246	0·278	$\gtrless$ IV + V IV = V = VI
	Segment IV	0·111	0 130	0 117	0·135	IV + V + VI = XII–XVI
	Segment V	0 112	0·130	0 117	0 135	Antennal formula $\frac{1}{\text{III–XV}}$
	Segment VI	0·111	0·130	0·120	0·135	
	Segs XII–XVI	0·345	0·370	0·360	0·430	= 1 5 × IIIrd
	Total length	1 600	1 743	1 670	1 914	= 6·8 × IIIrd, 4 6 × XII–XVIth
Palp	Segment 1	0·030	0·033	0·033	0 036	Formula, 1, 2, 3, 4, 5
	Segment 2	0 090	0·090	0·090	0·100	Relative lengths, 2 2, 6 1, 8·5, 10, 21 1
	Segment 3	0 120	0·135	0·123	0·141	= 1 + 2
	Segment 4	0 144	0·156	0 144	0·165	> 1/5th palpal length
	Segment 5	0·306	0 333	0·315	0 345	
	Total length	0·690	0·747	0·705	0·787	
Wing	Length	1 785	1 900	1 857	2 143	= 5 × breadth, 0·66 × hind leg
	Breadth	0·350	0·385	0·370	0·443	
	α	0·270	0·257	0·300	0·357	$\frac{\alpha}{\beta}=0{\cdot}75$ $\frac{\beta}{\gamma}=1{\cdot}27$
	β	0·357	0·393	0·364	0·457	
	γ	0·343	0·270	0 330	0·300	$\frac{\alpha}{\gamma}=0{\cdot}96$ $\frac{\delta}{\alpha}=0{\cdot}39$
	δ	0·100	0·100	0·114	0·164	
	ε	0 413	0·430	0 443	0·514	$\frac{\alpha}{\varepsilon}=0{\cdot}63$ $\frac{\theta}{\varepsilon}=2{\cdot}17$
	θ	0·900	0 970	0·928	1·100	$\frac{\alpha+\beta}{\theta}=0{\cdot}71$ $\frac{\text{Wing}}{\theta}=1\,97$
	—	0·100	0·114	0·107	0·114	

* Averages taken from four specimens

TABLE II—*concld*

	Structure	Lengths in mm of specimens number —				Remarks, average relative lengths, etc*
		1	2	3	4	
Hind leg	Femur	0·700	0·711	0·711	0·785	= ⅓ leg
	Tibia	0·961	0·970	0·970	1·111	= ⅓ leg, <2 × tarsus seg 1
	Tarsus, seg 1	0·500	0·500	0·500	0·571	
	Tarsus segs 2–5	0·614	0·657	0·643	0·714	
	Total length	2·80	2·84	2·85	3·2	(Not including coxa and trochanter)
Genitalia	Sup clasper, seg 1	0·276	0·290	0·285	0·300	= 2·3 × seg 2, 1·32 × intermed app
	Sup clasper, seg 2	0·120	0·132	0·126	0·135	
	Intermed append	0·207	0·222	0·210	0·231	
	Intromit organ	0·168	0·180	0·171	0·195	
	Inf clasper	0·216	0·210	0·222	0·255	= 1·7 × inter app, 1·14 × subgen lamellæ
	Subgen lamellæ	0·190	0·210	0·190	0·216	

*Averages taken from four specimens

clasper is unarmed and is slightly shorter than the basal segment of the superior clasper, but longer than the intermediate appendage It is about 1 14 times as long as the *subgenital lamellæ* The *intromittent organ* is narrow with a slightly dilated apex The *genital filaments* are protruded in some specimens, and have obliquely truncated tips The *pompetta* usually lies about the junction of the 6th and 7th abdominal segments, except in those specimens with protruded genital filaments in which it may be more posterior

The male of *P baityi* var *campesteri* resembles that of the type except that some specimens show a small but distinct solid-looking pigmented area (Plate L, fig 6)

Differential diagnosis

The morphology of the male genitalia is quite distinct from any of the members of the erect-haired group except *P hospitii*, *P christophersi* and *P clydei* The pigmented areas in these species is much larger than in *P baityi*, in addition *P clydei* has a palpal formula of 1, 2, 4, 3, 5 and in *P christophersi* the combined length of palpal segments 1 and 2 is less than that of 3

In the recumbent haired group the morphology of the male hypopygium of *P sylvestris*, *P zeylanicus* and *P himalayensis** is distinctive In *P minutus*,

*It was suggested in an earlier paper (Sinton, 1924) that the number of spines on the distal segment of the superior clasper of *P himalayensis* might be four, not three, as originally believed An examination of some new specimens of this species has shown that in addition to the three spines described in the type specimen, there is also a smaller fourth sub-apical one

P barraudi and *P squamipleuris* the spines on the superior clasper are all apical and the non-deciduous spine is more proximal The intromittent organ in *P minutus* is also broader and more rounded apically None of the spines on the superior clasper of *P babu* are so markedly sub-apical as in *P bailyi* and the non-deciduous spine is more proximal The plate joining the lateral bars of the buccal cavity of *P babu* is either very markedly concave on its posterior margin or shows a distinct notch, while in *P bailyi* it is almost straight The male genitalia of *P montanus* closely resemble those of *P bailyi* but the buccal morphology makes its differentiation easy (*cf* Sinton, 1927, Plate VIII, fig 11) The distribution of the spines on the superior clasper in *P malabaricus* is very like that in *P bailyi* but the very short size of β, the much longer IIIrd antennal segment, the broader wing and the 3rd palpal segment shorter than the combined length of 1 and 2 differentiates this species

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Idem (1927*a*) *Ibid*, pp 33–40

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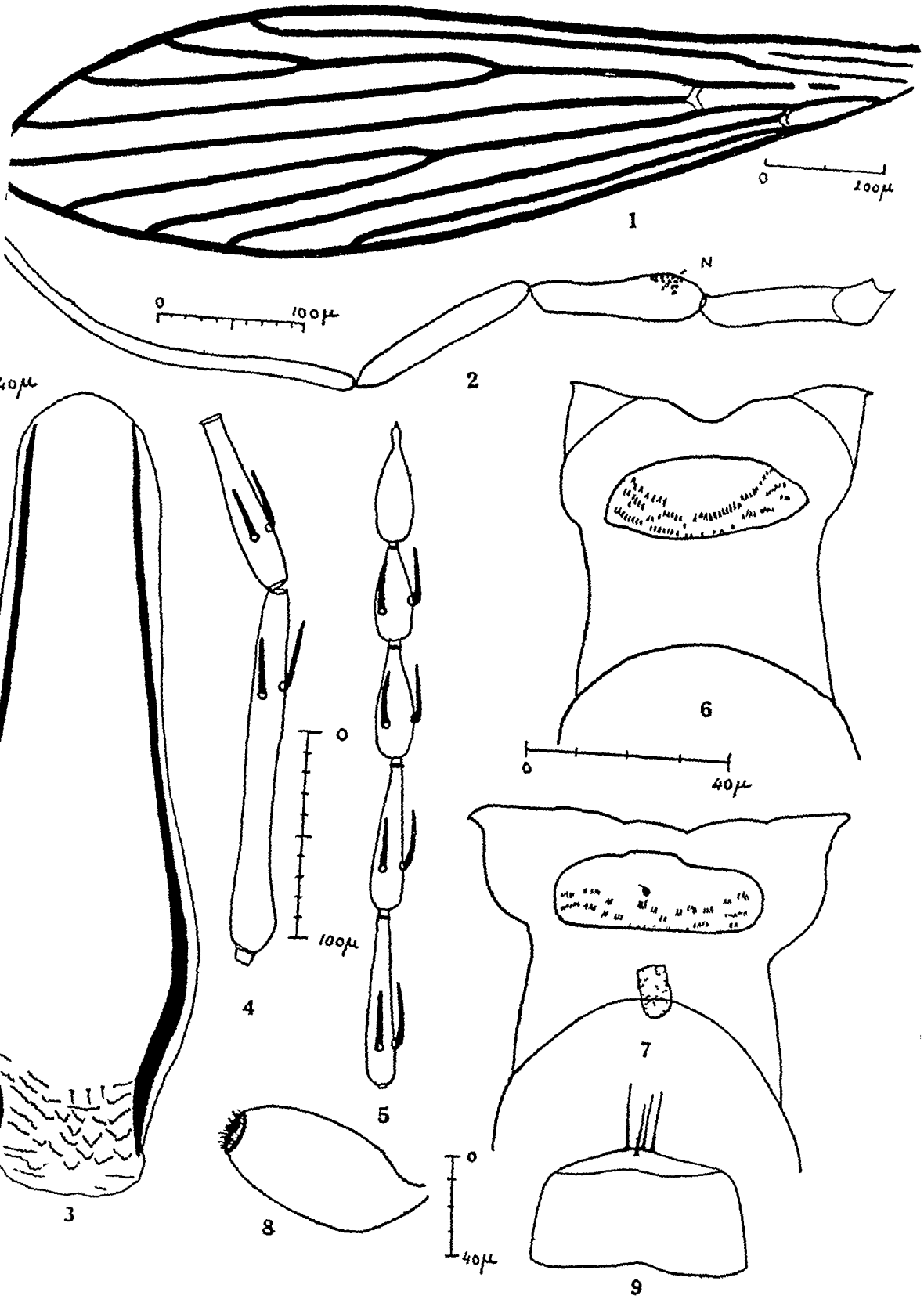
Explanation of Plate XLIX

Phlebotomus bailyi (♀)

Fig 1 Wing
„ 2 Palp N Newstead's spines
„ 3 Pharynx
„ 4 Antennal segments III and IV
„ 5 Antennal segments XII–XVI
„ 6 Buccal cavity of *P bailyi* (type)
„ 7 Buccal cavity of *P bailyi* var *campester*
„ 8 Spermatheca
„ 9 Post-genital plate

PLATE XLIX

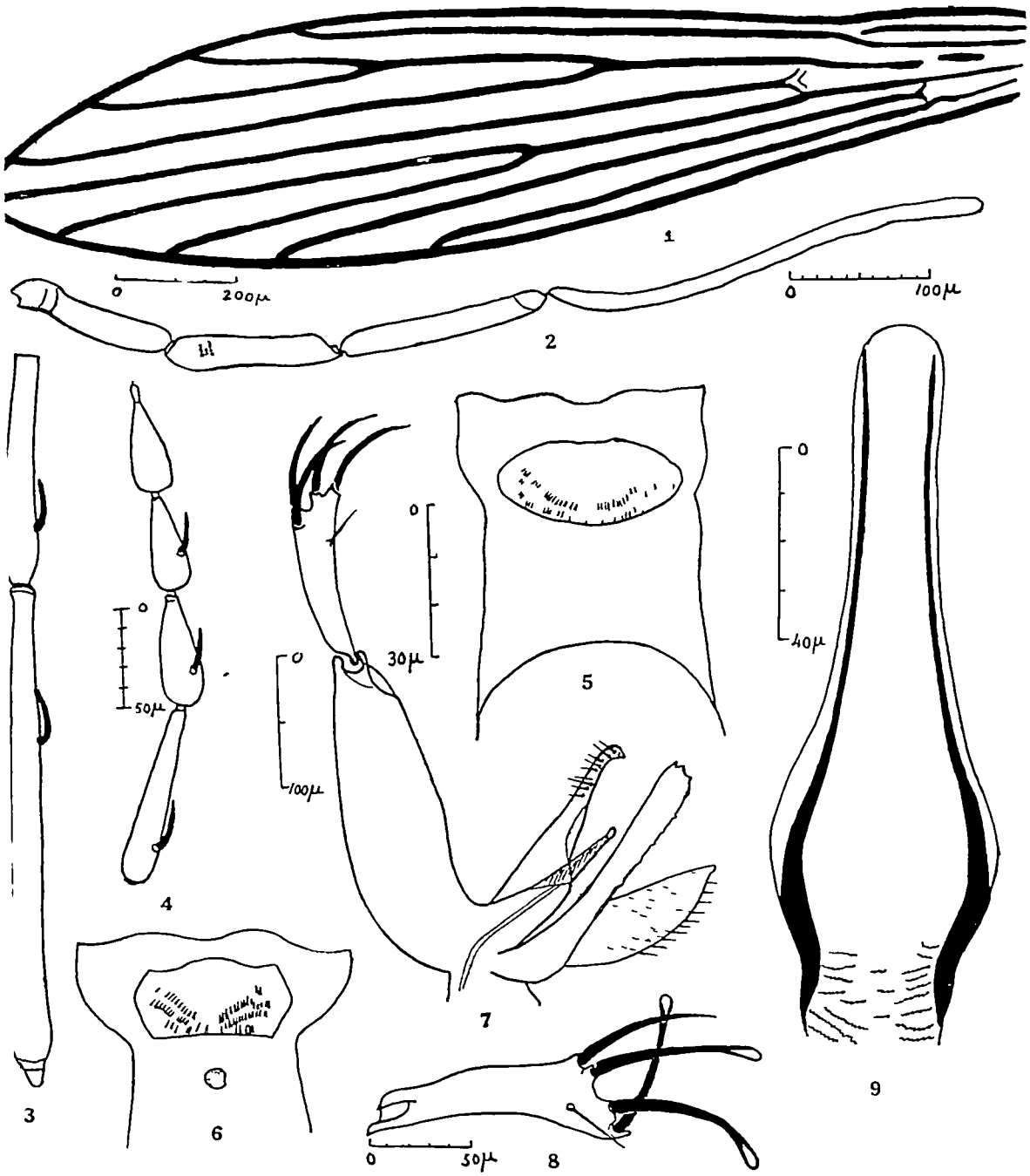


Explanation of Plate L

Phlebotomus bailyi (♂)

Fig 1 Wing
„ 2 Palp
„ 3 Antennal segments III and IV
„ 4 Antennal segments XIII–XVI
„ 5 Buccal cavity of *P bailyi* (type)
„ 6 Buccal cavity of *P bailyi* var *campester*
„ 7 Male hypopygium
„ 8 Distal segment of superior clasper
„ 9 Pharynx

Plate L



STUDIES IN MALARIA, WITH SPECIAL REFERENCE TO TREATMENT

Part XIV

THE EFFECTS OF DOSAGE OF DRUGS AND DURATION OF TREATMENT ON THE PRODUCTION OF CURE

BY

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A GREAT variety of opinion seems to exist as to the best dosage of quinine and the duration of treatment necessary for the cure of malaria These are points of considerable interest both to the physician and the patient As malaria is a common disease among the poor population of the tropics and the cinchona alkaloids are relatively expensive drugs, these points have also an important economic aspect

Our more precise knowledge of the relationship of dosage and duration of treatment to the production of cure, both clinical and permanent, depends mainly upon the extensive investigations on malaria which were carried out during the War and afterwards* In any discussion of these factors it is necessary to consider separately the palliative effects of the drugs on the clinical manifestations of the malarial fevers and their effects in the production of a permanent cure of these diseases

*Although a very large amount of literature has accumulated around the subject of quinine treatment, much of this is useless for comparative purposes Even when a similar dosage has been used by two different observers, the method of administration, the duration of treatment, the chronicity of the infection, the season of the year, the type of population, the criterion of relapse, etc, etc, have varied markedly in the different investigations It is only when these factors are more or less constant in any series of observations, and when proper scientific precautions have been taken in the work, that the results are comparable (Sinton, 1926)

(1) Palliative effects

As quickness of action is to be aimed at in the amelioration of acute attacks of these fevers, dosage in relation to rapid action is important The first point to be considered in such a discussion is whether any differences have been found between the effects of different doses in the treatment of infections with each of the three species of malarial parasites The palliative action of the different forms of treatment seems best evaluated by their effects on the duration of fever and the persistence of parasites in the peripheral blood

(a) Dosage in relation to the species of parasite

It has been the experience of clinicians for many years that larger doses of quinine seemed necessary for the control of the clinical manifestations of malignant tertian malaria than of benign tertian Waters (1912) and Bass (1922c) found the clinical symptoms of most cases of benign tertian malaria relieved by relatively small doses of quinine, but the majority of malignant tertian cases required a much larger amount This has also been the experience in our Kasauli work The work on the infections with *P vivax* for therapeutic purposes have demonstrated that these are amenable to very small doses of quinine

MacGilchrist (1915) determined the minimal lethal doses of quinine for the vulnerable stages of *P. vivax*, *P falciparum* and *P malariæ* to be respectively about 0 10, 0 15 and 0 20 grm per 70 kilos body-weight of patient He also found that with equal doses of the cinchona alkaloids the average duration of fever was shortest in benign tertian and longest in quartan, with malignant tertian intermediate Eugling (1918) reports that in Albania a single dose of quinine less than 0 75 grm for an adult was insufficient to damage or diminish considerably the number of benign tertian parasites in the peripheral blood, while at least 1 0 grm was required in malignant tertian cases Stephens *et al* (1917) showed that a single intravenous injection of 10–15 grains of quinine would cause a clinical cure in benign tertian but not in malignant tertian malaria

Fletcher (1925) tested the effects of quinine and quinidine sulphate in the treatment of malaria in Asiatics The dosage was regulated in proportion to body-weight The patients all suffered from a mild form of the disease and there was an unusually large proportion of quartan infections The average number of doses of quinine required to cause a disappearance of parasites from the peripheral blood was 8 7 in quartan, 4 5 in benign tertian and 6 6 in malignant tertian infections With quinidine the results were 9 1, 3 4 and 4 3 doses respectively The average number of doses of quinine needed to abolish fever was 3 5 in quartan, 4 8 in benign tertian and 4 7 in malignant tertian cases, while with quinidine they were 4 7, 2 4 and 3 5 respectively

These reports, combined with the very extensive clinical evidence, indicate that the minimal dosage of quinine needed to control the clinical manifestations

of benign tertian malaria is less than that needed in malignant tertian infections The work of MacGilchrist (1915) and of Fletcher (1925) also suggest that an even greater dosage may be required in quartan malaria

(b) Relation of dosage to the cure of clinical symptoms

A great variety of opinion exists as to the correct dosage of quinine to be used during the acute attack of malaria, but, as was seen in the discussion above, this probably depends upon the species of parasite responsible for the infection being treated

In malignant tertian malaria Thomson (1917) considers that nothing less than 30 grains daily is sufficient and even as much as 45 grains may be given Rogers (1918) recommends 30–45 grains daily during the acute stages of the disease Deeks (1925) in primary infections gives a daily dosage of either 30–60 grains daily for a short period or 5–10 grams for a longer time In the West Indies and in Panama most clinicians seem to recommend a dosage of 45 grains daily during the acute stages of malignant tertian malaria, indeed in most areas in the tropics where this fever is common, 30 grains seems to be the minimal daily dosage recommended to control the fever

Marshall (1918) states that quinine 'must be given in adequate doses, at least 30 grains in 24 hours, small doses are not only useless but may be harmful' Ross (1919) says 'moderate doses of quinine – say between 20 and 40 grains daily for adults – suffice in the vast majority of cases to reduce both fever and asexual parasites within two or three days' In an Editorial in the *Indian Medical Gazette* (1921) 20 grains is given as the minimum effective dose of quinine Bass (1922*b*) recommends 30–40 grains daily during the acute stages Platt-Johnson and Gilchrist (1921), in a series of malaria cases (61 per cent benign tertian), found that, as the daily dosage of quinine rose from 16 to 23 grains, there was a fall in the average number of days during which it was necessary to keep patients in hospital In benign tertian malaria Ross (1921) considers that doses greater than 15 grains daily are only necessary during fever

Fletcher (1923) conducted a series of carefully controlled experiments in the Federated Malay States He found that doses as small as 10 grains of quinine daily were enough to cure the attack in benign tertian, malignant tertian and quartan malaria in Asiatics, who weighed on an average about 100 lb, but considers that for routine treatment a dosage of 10 grains twice daily should be used *

The long series of investigations, carried out by Stephens and his colleagues at the Liverpool School of Tropical Medicine from 1917 to 1919, produced

* This daily dosage would correspond to almost 30 grains for a European weighing about 10 stone It must also be remembered that these Asiatics had lived most of their lives in malarious areas and may thus have developed a certain degree of tolerance to the clinical effects of the infection, thus reacting more easily to smaller doses of quinine

some very interesting results in the treatment of chronic benign tertian malaria among European patients (*a*) When graduated doses of quinine from 5 to 90 grains were given on each of two successive days, it was found that it usually required a daily dosage of 10 grains or more to cause a cessation of febrile paroxysms (Stephens *et al*, 1918) (*b*) The palliative effects of continuous daily doses of 30 and 15 grains were more marked with the larger doses (Stephens *et al*, 1918*a*) (*c*) The palliative effects of two large doses of quinine given on two consecutive days weekly were better than the same total amount spread over the week in small divided doses (5–15 grains), and a total of 90 grains weekly was better than 30 (Stephens *et al*, 1919)

From a general study of the enormous amount of literature on the treatment of malaria, it would seem a general rule that, in those areas where *P vivax* is the common parasite and *P falciparum* rare, doses of 20 grains of quinine daily are usually considered adequate In the tropics, however, and more especially in those regions where *P falciparum* is the predominant parasite and where such infections may assume a pernicious character, 30 grains of quinine seems to be the minimum daily dosage usually recommended during the acute stage of the malarial fevers Workers in the latter areas have often to depend in practice upon a clinical diagnosis, for neither the time nor the facilities may be available to make an accurate diagnosis of the species of parasite responsible for the fever in all cases They also realize that mixed infections may not be detected in ordinary microscopical examinations, therefore, in their routine dosage they legislate for the more severe infection, *i e*, malignant tertian malaria

In the investigation of nearly 4,000 cases of malaria carried out during the last 8 years in Northern India, we have always found that a daily dosage of 30 grains of quinine, if properly administered, was effective in controlling the acute clinical symptoms of malaria, in the absence of any complicating disease

The conclusions arrived at from our experience and from the above evidence are that (*a*) while 20 grains of quinine may be sufficient to control the acute manifestations of benign tertian malaria, yet a more rapid action is probably obtained with a daily dosage of 30 grains, (*b*) a daily dosage of not less than 30 grains of quinine is probably required during the acute stages of quartan and malignant tertian infections and (*c*) for routine work 30 grains of quinine daily seems the best daily dosage in the acute stages of malaria, irrespective of the species of parasite involved

(*c*) *Effects of dosage on the persistence of parasites in the peripheral blood*

MacGilchrist (1915) tested the effects of both large and small doses of the different cinchona alkaloids on the three forms of malarial infection and found that the rate of disappearance of parasites from the peripheral blood was more rapid with large doses than with small ones Platt-Johnson and Gilchrist

(1921) found that the positive blood findings after 8 days of treatment fell from 29 per cent with a daily dosage of 18 grains of quinine to 19 per cent when 27 grains were given

The following results with chronic benign tertian malaria were obtained by the Liverpool workers (*a*) Daily doses of 10 grains or more of quinine on two consecutive days caused the disappearance of all stages of *P vivax* from the peripheral blood, but with doses of 5 grains this was not always so (Stephens *et al*, 1918) (*b*) Continuous doses of 45 grains daily were more effective than 30 grains (Stephens *et al*, 1918*a*)

These results support the view that large doses have more effect than small ones in causing a disappearance of parasites from the peripheral blood This is in keeping with the results obtained in the amelioration of clinical symptoms, which have been discussed in the previous section

(*d*) *The effects of daily dosage on the occurrence of relapses during treatment*

Thomson (1917) found that doses of 5 grains of quinine daily were insufficient to keep under control the symptoms of malignant tertian malaria Bass (1922) says that this dosage is not sufficient to kill the parasites and cure the infection in all cases of malaria Ross (1921) states that '8 grains or more in benign tertian malaria sufficed almost invariably to improve the cases greatly while being taken' Bass (1922*b*) thinks that 'there are practically no cases that continue to have parasites in their blood while taking as much as 10 grains of quinine sulphate by the mouth daily following the relief of the active symptoms' Treadgold (1918) reports that parasites could be found in the blood of 66 per cent of patients, mostly benign tertian cases, ordered 15–20 grains of quinine daily under War conditions, while they were found in only 50 per cent of those ordered 30 grains daily *

Fraser (1919), from a study of about 8,000 patients mostly suffering from benign tertian malaria, concluded that 'no man can relapse during the period he is swallowing 15 grains of quinine sulphate daily,' that smaller doses are not sufficient and that this amount is better given in a single daily dose Fletcher (1918) observed over 2,000 patients in the Federated Malay States, of whom about one-sixth were benign tertian infections and the remainder either malignant tertian or quartan Among these he only found 8 cases of relapse while a daily dosage of 20 grains of quinine was being given and in even these cases he suspected that the drug was being evaded Row (1919) recorded only 4 relapses among 774 European patients with benign tertian malaria given 10–20 grains of quinine daily, and even then he could not exclude the possibility that these patients were avoiding their medicine

* It must be remembered that under the stress of such conditions, it was much easier for patients to evade their medicine, and at the same time gastro-intestinal derangements were not an uncommon cause of defective absorption of the drug

Stephens *et al* (1918*b*) tested the effects of the oral administration of quinine sulphate in doses of 10, 15, 30 and 45 grains daily for two consecutive days weekly over long periods From the number of febrile parasitic relapse observed, these workers state that 'as a general rule, the smaller the dose the greater the number of relapses, this is well seen in the grains 15, 30 and 45 series, the figures given in the grains 10 series are anomalous, but their value must be to a certain extent discounted owing to the relatively small number of cases observed' Ross (1918) gives the results of several series of chronic benign tertian cases treated at Epsom during the War If his figures are examined it is found that the percentage of parasitic relapses during continuous treatment with 5 grains of quinine daily was probably about 16 5, with 10 grains about 3 2 and with 15 grains about 3 0 In two series treated at Oxford with 10 and 20 grains daily the figures were about 5 8 and 1 17 per cent Bass (1922*c*) states he 'has never been able to discover a case which continued to have symptoms while taking 30 grains or more daily'

Among over 600 cases of chronic benign tertian malaria investigated in our inquiry, when the temperature had been brought to normal by larger doses, no relapse, either clinical or parasitic, could be detected during the period while a daily dose of 10 grains of quinine in solution was being taken In many instances the treatment lasted 6 weeks

From the results recorded above it seems evident that daily doses of less than 10 grains of quinine in solution are in many instances insufficient to prevent relapse while being taken

(*e*) *Conclusions*

The evidence detailed above indicates that 20 grains of quinine daily for an adult is the minimal dosage which can be expected to produce the most rapid clinical action during the acute stages of malaria While this dosage may be sufficient in most cases of benign tertian malaria, 30 grains daily seems to be the minimal daily dosage for routine use in the acute stages of malignant tertian and quartan malaria

(2) Production of a permanent cure

In the production of a permanent cure in any form of malaria the duration of treatment seems to be intimately bound up with the size of the daily dosage of quinine used, and thus with the total amount of alkaloid taken In malignant tertian malaria little difference could be found by us between the cure rates produced in fresh as compared with chronic infections It is, however, almost universally recognized that in infections with *P vivax* the cure rate in these two conditions may vary very considerably under the same forms of treatment (Sinton and Bird, 1929)

(*a*) *Duration of treatment in relation to relapse*

Barlow (1915) records the results of varying durations of treatment in the production of permanent cures in nearly 600 cases of acute malignant tertian

malaria His treatment consisted of 20 to 30 grams of quinine bisulphate daily in capsules for 2 days, followed by 15 grams daily for one month and then 15 grams twice weekly for two more months He reports the following results —

Duration of treatment	Number of patients	Relapses Per cent
Less than one month	116	100
One month	246	37
Three months	218	*nil*

Cardamatis (1918) tested the value of prophylactic quinine on cases experimentally infected with *P falciparum* by mosquito bites He found that doses of 15 grams of quinine daily for one week failed to prevent infection in 33 per cent of cases, while doses of 6 grams daily with 9 grams every third day, when given throughout the summer, were successful Thomson (1917), as the result of his investigations of malignant tertian malaria, believes that prolonged courses are more effective than are short ones Chopra (1922) considers that courses of one month's duration are needed in malignant tertian malaria to effect a permanent cure, while in benign tertian malaria the duration must be two months Among the malignant tertian infections treated with quinine and alkali by Sinton (1926*a*), it was found that the relapse rate fell as the duration of treatment was increased from 4 to 7 days and with it the total amounts of quinine

Several workers have expressed opinions on the effects of the duration of treatment on the permanent cure of malaria, without any special reference to the species of parasite involved Bass (1922*a*) says that when the clinical symptoms have been abolished by doses of 30 grains or more of quinine daily, 'if we should give 10 grains daily to a group of malaria-infected persons for only one week and stop, a small proportion will be disinfected by even this short treatment, but most of them will relapse sooner or later If the treatment is continued two weeks a larger per cent would be disinfected If it is continued for four weeks 60 to 70 per cent are disinfected If it is continued for eight weeks between 90 and 95 per cent are disinfected It would probably take more than three months' treatment to disinfect 100 per cent' Baermann (1923) treated a number of patients suffering from infections with the different species of malaria parasite and records a relapse rate of 25 per cent following 30 grains of quinine daily for 4 months, as compared with 50 per cent when the course lasted 2 months Fletcher (1923) also thinks the duration of treatment as important as dosage and says 'with reference to giving reduced doses or interrupted treatment, towards the end of a course, we find ourselves in full agreement with Thomson (1917) that it is much better to continue with doses which are known to be curative rather than to play with the parasites by half killing them with the drug and then allowing them to recover'

Acton, Curjel and Dewey (1921), as a result of their work on chronic malaria, concluded that 'by increasing the duration of treatment of benign

tertian infections the chance of cure is relatively increased, thus it is 28·9 for a two months' course, 45·1 per cent for a four months, 61 per cent for a six months and 69 per cent for eight months'

Yorke and Macfie (1924) carried out some very valuable work on the 'prophylactic' use of quinine in 31 fresh infections with *P. vivax* produced by mosquito bite. They concluded from their results that any action of the drug was rather in the nature of an early cure than of a true prophylaxis by the destruction of sporozoites. They found that daily doses of 10 grains of quinine had to be continued for at least 10 days after the infecting bite to prevent the development of infection. Shorter periods were not sufficient, but with larger doses (30 grains) the period necessary was slightly shortened. Rudolf (1927) found that only 1·4 per cent of cases of inoculated malaria relapsed after a treatment consisting of a total of 200 grains of quinine administered during 17 days, while those patients who received a single dose of 20 grains of the drug all relapsed. He thinks that these results 'show clearly that the occurrence of relapses is dependent upon whether a very small amount of quinine is given or not'

In experimental infections of benign tertian malaria following mosquito bites, Yorke (1925) records a relapse rate of 57 per cent after a treatment of 30 grains of quinine daily for 3 days, while James (1926) reports a rate of only 5 per cent when a similar dosage was continued for 5 days. These two series of experiments were, of course, carried out under different conditions and possibly with different criteria of relapse, still a comparison of the results is interesting.

The results recorded by Sinton and Bird (1929, Tables II and III) following the treatment of a mixed population of both fresh and chronic infections with *P. vivax*, also indicate the possibility of a rise in cure rate in this fever as the duration of treatment rose from 4 to 7 days.

Yorke (1925), basing his conclusions mainly on the results obtained in the treatment of chronic benign tertian malaria, thinks that 'there is now overwhelming evidence that, within broad limits, the amount of the daily dose of quinine, the length of time it is continued, and the manner of administration do not exert any influence, either on whether or not a relapse occurs, or on the time it will occur'. Nicol (1927) also states that from his experience no known method of quinine treatment either during or after the attack is effective in preventing relapses in induced malaria.

The following table shows the results of the treatment of 875 patients suffering from chronic benign tertian malaria. The drugs used were quinine sulphate or quinidine sulphate in solution by the mouth. It will be seen from these that in our work also there was found a definite relationship between the duration of treatment and the cure rate. The one anomaly in this table may have been due to the fact that the treatment was an interrupted one.

TABLE

Alkaloid	Treatment Grains of drug × days of treatment *	Total drug (grains) — total days of treatment	Total number of patients	Number lost sight of	Number not relapsing	Number of relapses	Calculated average percentage of relapses
Quinine	24 × 10	240 — 10	103	7	21	75	76·8
	20 × 1 30 × 6 T × 7 20 × 7	340 — 14	124	2	19	103	84·0
	30 × 14 20 × 7	560 — 21	186	38	44	104	67·3
	30 × 7 T × 7 30 × 7 10 × 35	770 — 49	82	4	31	47	60·0
	30 × 14 10 × 42	840 — 56	172	15	69	88	55·6
Quinidine	20 × 7 T × 7 20 × 7	280 — 14	97	1	13	83	86·2
	20 × 21	420 — 21	81	5	12	64	82·8
	21 × 7 14 × 24	483 — 31	14	8	0	6	76·4
	20 × 28	560 — 28	16	0	5	11	68·7

* Indicates a course of iron and arsenic tonic but no quinine

From the work quoted above it is concluded that the cure rate in malaria rises with the duration of the quinine treatment, when the drug is given in medicinal doses In chronic benign tertian malaria, this increase in rate is not directly proportionate either to the length of treatment or the total amounts of quinine given

(b) Dosage in relation to permanent cure

It would seem that, within certain limits, the dosage is of less importance than the duration of treatment The exact limits of such dosage and the effects of smaller or larger doses must be considered

Ross (1918) says that 'success seems to vary directly with the magnitude of the daily dosage' Deeks (1925) found in Panama, where malignant tertian infections are very common, that daily doses of 30 grains of quinine continued until the temperature had fallen to normal for one or two days were followed by many relapses but, when 45 grains daily was given till the fever disappeared and doses of 30 grains continued for 5 days afterwards, there was a great diminution in relapse rate This worker 'favours the conclusion that in the treatment of primary infections heroic doses (30 to 60 grains) of quinine should be given for the first two or three days and reduced doses for an additional week, or if small doses (5 to 10 grains daily) are given the treatment should be continued for from ten to fourteen days longer to effect a cure'

Stephens *et al* (1918), using a daily dose of 5 to 30 grains of quinine for two consecutive days in chronic benign tertian malaria, record a relapse rate of 98 per cent in 68 cases, with 45 grains daily in 12 cases, 75 per cent, with 60 grains daily in 12 cases, 58 per cent and with 90 grains daily in 76 cases, 38 1 per cent They concluded that 'if the dose given on each of the two days does not exceed 30 grains no curative effect is obtained when the dose given on each of the two days reaches 45 grains or more a curative effect is manifest, this becomes more marked as the dose increased from grains 45 to grains 90' These workers (Stephens *et al*, 1918*a*) tried continuous treatments of 20, 30 and 45 grains daily for several weeks and found of these that of grains 45 was best The conclusions of Stephens *et al* (1918*b*), that larger doses were more effective in preventing parasitic relapses during treatment, have already been quoted These workers, however, found little difference between the permanent curative effects of 30 and 90 grains given weekly for 2 months, whether administered as two large doses or in small doses daily (Stephens *et al*, 1919)

Caridamatis (1918*a*) reports from the observation of 480 cases of malaria that 15 grains of quinine daily for 15 days produced a 'radical cure' in 40 per cent of malignant tertian infections, in 45 per cent of quartan and 55 per cent of benign tertian In another series of 144 cases treated with 22 grains of quinine daily for 12 days, the percentages were 88, 70 and 87 respectively Ross (1921) states that 'even treatments commencing with 100 grains a day and continued for nearly a month in smaller doses gave no guarantee of permanent cure, though they seemed to yield slightly better results on the average than did smaller scales of dosage' Anderson (1922) tested the value of daily doses of 20, 45 and 60 grains of quinine and reported that the relapse rate fell as the daily dosage of quinine was increased

The results recorded in the Table show that, under the conditions of our experiments, as the total dosage of the cinchona alkaloids increased so did the cure rate in chronic benign tertian malaria

The investigations recorded above indicate that better effects in the production of a permanent cure are obtained with larger doses of quinine than with smaller ones It would also seem that within certain limits of dosage, the

duration of treatment as well as the total amount of the drug given, has also an important influence

While daily doses of from 20 to 30 grains of quinine have been found by most clinicians sufficient to control the clinical manifestations of malaria and produce a permanent cure in a great many cases, yet other workers have at different times reported that such doses have been ineffective in these respects and that they have not eradicated the parasites from the peripheral blood In consequence of this doses as high as 60 to 90 grains daily, or even more, have been recommended for use in the acute stages of the disease

The so-called failure of oral quinine in doses of 30 grains daily to produce a clinical cure in some instances may have been due to a variety of causes (*a*) the medicine ordered was not taken or the proper quantities were not swallowed, (*b*) if taken, the strength of the mixture was not that ordered, (*c*) the dose was not retained, either voluntarily or involuntarily, (*d*) the drug was not absorbed, due either to gastro-intestinal disturbances or to being given in a form which prevented complete absorption (Sinton, 1925, 1926) During the controlled treatment of 1,505 cases of malignant tertian malaria and 1,873 of benign tertian, carried out in our investigations during the last 9 years, it has never been found necessary to give quinine, or any of the other cinchona alkaloids, in doses larger than 30 grains daily in solution by the mouth, to cure an acute attack of malaria, when steps were taken to eliminate the fallacies mentioned above (Sinton, 1926)

Doses as large as 45 grains daily can usually be tolerated by the average adult for a few days, but this amount seems about the ordinary limit of tolerance in most cases This is the dosage which has been recommended by most physicians in the cases of severe malignant tertian malaria seen in Central America The effects of very large doses of quinine were tested at Liverpool, where Stephens *et al* (1918*a*) found that continuous treatment with 20 to 30 grains daily was tolerated for 8 weeks, but only 12 out of 19 patients could stand 45 grains daily for this time The same workers (Stephens *et al*, 1918) report severe cinchonism with 90 grains daily for 2 days and found that 5 out of 15 patients could not tolerate doses of 120 grains daily for the same period (Stephens *et al*, 1918*c*)

It is well known that excessive doses of quinine may have a harmful effect on different organs of the body Nierenstein (1919) found that when the concentration of quinine in the urine rose above a certain point, that 'complications apparently set in which produced a passing albuminuria' This worker and others report that medicinal doses of more than 30 grains of quinine orally are liable to produce this effect and that even smaller doses by the intravenous or intramuscular routes may cause it

In the experiments of Stephens *et al* (1918*c*) mentioned above, the relapse rate was greater following daily doses of 120 grains of quinine than with 90 grains, but the number of cases treated were too few from which to draw any definite conclusions in the matter McCallison and Cornwall (1918)

suggest that massive doses cannot fail to retard 'the development of that natural immunity on which the cure of the disease depends' Ross (1921) thinks that heroic doses may have less effect on the parasite than moderate ones and considers that anything over 15 grains daily is only required when fever is present Yorke (1925) also suggests that 'there may be a danger from excessive dosage, or from prolonged administration of quinine, of exhausting the body cells which react with quinine to form the parasiticidal substance,' and thinks that this may be the reason why War cases were so difficult to cure

It would seem from these results that an excessive daily dosage of quinine may have an injurious effect on the body and it suggests that such doses may tend to defeat their own object when used to produce a permanent cure

(c) *Conclusions*

The results detailed above show that by a more prolonged course of treatment the permanent cure rate can be increased, but that the increase is not directly proportional to the duration of treatment or to the total amount of quinine given

The evidence also indicates that better effects in the production of a permanent cure can be obtained by larger doses of quinine than by smaller ones It seems probable, however, that the continued use of large doses over long periods may have a harmful effect on the human body, and may even hinder the process of permanent cure

(3) Discussion of the relationship of the results to the routine treatment of malaria

The results recorded previously favour the view that for the routine treatment of malaria doses of, at least, 30 grains of quinine daily are required to produce the most rapid clinical effects during the acute attack In our experience when proper precautions were taken to ensure absorption, a dosage of 30 grains of quinine in solution daily was found efficacious in all cases It seems possible that, in those instances where larger doses have been recorded as necessary, the patient was not absorbing the drug completely due either to some gastro-intestinal condition or because the drug was given in a form which did not permit of complete absorption While doses of 20 grains daily may suffice in most cases of benign tertian malaria, it is probably insufficient to produce the best clinical results in malignant tertian and quartan malaria A daily dosage of 30 grains of quinine in readily absorbable form would seem to be effective This dosage would avoid the possible clinical inefficiency of smaller doses and the possibly harmful and certainly disagreeable effects of larger doses

In the production of a permanent cure 30 grains daily seems to be the maximum daily dosage which can be tolerated by most adults for periods of more than a few days at a time

Having decided that 30 grains of quinine daily is the optimum daily dosage, for routine treatment, one must now consider what is the optimum duration of treatment The work quoted above shows that with more prolonged treatment the number of relapses can be reduced The results obtained by Sinton (1926*a*) show that one week of treatment with quinine and alkali will cure a very large percentage of malignant tertian infections It has also been found that a very high percentage of fresh infections with *P vivax* can be cured by short courses of quinine (Sinton and Bird, 1929) The instances where high relapse rates have been reported after prolonged courses of quinine, properly administered, have almost invariably occurred when a selected population of chronic benign tertian infections was being treated These chronic cases have been estimated by Wright (1922) to form only about 10 per cent of all patients Under such conditions one does not seem justified in subjecting all patients to the discomfort and expense of very prolonged courses in an attempt to procure a slightly higher percentage of cures It would seem more reasonable to treat all primary infections with known curative doses (30 grains daily) for a short period (one week) and ensure that during that time the treatment is taken, retained and absorbed in the doses prescribed Such treatment if properly carried out should cure at least 70 per cent of fresh malarial infections, and so a very large number of patients would be spared the disadvantages, both pecuniary and bodily,* which prolonged administration entail The smaller percentage of relapse cases could then be dealt with some of the special methods suitable for such cases

A suggested routine treatment of malaria based on these lines has been published (Sinton, 1930)

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STUDIES IN MALARIA, WITH SPECIAL REFERENCE TO TREATMENT

Part XV.

DOES THE STRAIN OF PARASITE INFLUENCE CURE?

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IF one neglects for the moment the effects of any medicinal treatment, one of the chief factors in determining the severity of the clinical manifestations of any infection and the chances of recovery, either clinical or permanent, is the virulence or toxicity of the pathogenic organism involved The severity of such infections may be augmented by an increase in the inherent virulence of the organism, by a low degree of resistance in the host, by increased dosage of the organism or by a combination of these factors

As judged by the experiments on virulence which have been carried out with certain of the pathogenic blood protozoa, this is a factor which may have an important bearing upon the mechanism of cure in malaria It is a well-established fact that different strains of the same protozoon may differ markedly in their virulence to animals Such differences have been studied more especially with trypanosomes and piroplasms It has also been proved that the virulence of the same strain of some of these protozoa can be markedly enhanced by a series of passages through certain animals, while passage through another species of animal may lower the virulence The normal degree of virulence can, however, be re-established by repeated passage through other susceptible animals

Unfortunately animal investigations of this nature have not been carried out in the case of the human malarial parasite, because so far none of the common laboratory animals have been found susceptible to infection with this parasite The recent work on the experimental infection of patients suffering

from mental diseases with *P vivax* for therapeutic purposes has, however, produced some very interesting observations which have a bearing on this subject

The virulence of any strain of parasite may be manifest by the toxic symptoms it produces in the host, and this influences the degree of ease or difficulty with which a clinical cure can be produced either by natural or medicinal means But it may also be manifest by the power of the parasite to resist such means and to survive in the host, in spite of all attempts to eradicate it and so produce a permanent cure of the disease

The effects of parasitic invasion may be considered under the following headings (*a*) The inherent or normal virulence of different strains of parasite, (*b*) any enhanced virulence due to temporary conditions and (*c*) the dosage of the parasite received by the host

(*a*) *Variations in the normal virulence of different strains*

The work on therapeutic infections with *P vivax* suggest that the difference between apparent resistance to infection or to its clinical manifestations and infection with marked clinical symptoms is only a matter of degree, all stages intermediate between these two extremes being found

Kirschbaum (1917) reports that in therapeutic malaria he has found no evidence of differences in the virulence of the strains of *P vivax* used by him, although the geographical origin of these was very diverse He attributes the different clinical effects observed to individual differences in the hosts On the other hand, the observations of some workers (Rudolf, 1924, Pijper and Russell, 1925, Bunker and Kirby, 1925, Lilly, 1925) indicate that different strains of *P vivax* may vary very considerably in their virulence, as judged by the severity of the clinical symptoms produced

Patients who have proved refractory to one strain of this parasite have been found susceptible to another (Nicole and Steel, 1926), or even to the same strain after passage through the mosquito (Rudolf, 1927) Rudolf (1927) suggests as the result of his work, that 'the question of the degree of immunity produced by artificially-inoculated malaria varies with the strain of parasite employed' James and Shute (1926) managed by using a different strain of *P vivax* to infect, by means of mosquitoes, some cases which had previously been found resistant to blood inoculation with another strain These workers draw the conclusions that 'several artificial inoculations, at intervals, by the direct-blood method of one strain of *P vivax* do not confer an immunity against mosquito infection with another *vivax* strain' They also conclude from their work that one attack of malaria (induced either by blood inoculation or mosquito bites) due to a strain of *P vivax* does not confer an immunity against a second infection with the same strain' but point out that 'the clinical character of the second infection is quite different from a primary infection'

Marchoux (1922) thinks that there is a multiplicity of strains of the three malarial parasites This, he considers, accounts for the records that Senegal

negroes immune to malaria in their own country succumb to the disease when taken to Dahomey Legeir and Nogue (1923) have also brought forward epidemiological evidence in support of the view that the inhabitants of an area may become tolerant to the local strain of parasite, yet at the same time be susceptible to the pathogenic effects of strains present in other areas Boeckh (1925), Ciuca, Ballif and Vieru (1928) and Dschaparidse (1929) think that immunity to malaria differs with the different species of malaria parasite, while the first worker believes that it varies also with different strains of the same parasite

Several workers have put forward as an explanation of the severe type of malaria seen in some regions during the War, that there had been introduced by the troops from other areas a quinine-resistant strain of parasite * Plehn (1927) reports such a strain of *P falciparum*, which persisted during three passages through patients being treated for general paralysis of the insane, but found that while the parasites resisted quinine, this resistance had no relationship to the toxicity of the parasite Sergent and Sergent (1921) have also reported a quinine-resistant strain, which developed among their birds in Algeria Arguing from his observations Plehn (1927) considers that the severe malaria of the War can be explained by the development of such quinine-resistant strains, which maintained their peculiarity through several passages He suggests that the quinine-sensitive strains would be killed off before the formation of gametocytes by the intensive quininization in force, while the resistant strains would survive and produce gametocytes, thus being spread by the mosquito and becoming the predominant form Ziemann (1920) also thinks that malarial parasites may be divided into avirulent forms, virulent forms and intermediate forms, according to their degree of resistance to quinine medication Wenyon *et al* (1921) have put forward the hypothesis that the failure of quinine prophylaxis during the War may have been due either to certain sporozoites or young forms arising immediately from them, being resistant to quinine

If such quinine-resistant forms exist, as judged by the failure of quinine, properly administered, to cure clinical symptoms and cause a disappearance of parasites from the peripheral blood, they must be extremely rare, at least, in Northern India An exhaustive search for such strains among nearly 4,000 cases of malaria, carefully investigated during the last 8 years, has failed to reveal a single resistant case, in spite of the fact that more than half the patients had previously been subjected to many and prolonged courses of quinine treatment

There are, however, other possible factors which may explain the severity of the symptoms during the War and the chronicity of the infections which

* The term 'quinine-resistant' is used in our paper to denote those cases in which it is reported that the parasites in the peripheral blood or the fever persisted in spite of adequate quinine treatment It is not applied to those cases in which a clinical cure is produced but which relapse at a later date when treatment has terminated

reached the base hospitals The possibility of the introduction of multiple foreign strains of parasite, not necessarily quinine-resistant, from other regions must be considered The troops engaged in malarious areas had, in many instances, already been in other malarious countries, and in this way, if strains of the parasite which differ in their effects on the human host do exist, the chance of the introduction of such strains was almost certain

It is well known in many malarious areas, where large numbers of troops were crowded together, that the individual was liable not only to one bite from one infected mosquito but to numerous bites from innumerable mosquitoes Under such conditions the chance of a person receiving an infection with one or more strains of parasite of varying degrees of virulence was considerable Such virulence may have been of a nature which caused more severe clinical manifestations or which was more resistant to permanent cure The fact that infection with one strain of parasite does not necessarily produce immunity to the clinical effects of another (James and Shute, 1926, James, Nicol and Shute, 1929), would also mean that with infection by each new strain the sufferer would be liable to a recurrence of clinical manifestations of greater or lesser intensity

Bates (1912) does not believe that strains of parasites inherently resistant to quinine exist nor that strains of different virulence occur in different countries Yorke (1925) also thinks that the strain of parasite had little or no effect on the causation of relapse, although in the theory of cure formulated by Yorke and Macfie (1924) they speak of the development of immune-body resistant strains Yorke (1925) states that 'observations made on War cases did not support this hypothesis (i e, of different strains), as the vast majority of patients from such different regions as Gallipoli, Salonica, Mesopotamia, India and Africa relapsed' One must, however, remember in this connection that the vast majority of malaria infections contracted during the War were treated in the field and that usually only those patients who relapsed on many occasions found their way to base hospitals, from which if still uncured they were repatriated to Great Britain or other non-malarious places It was at these places that most of the careful scientific observations on the permanent cure of the disease were carried out During the numerous courses of treatment which these patients must have received before reaching their final destinations, it seems only reasonable to suggest that, if differences in strains do exist, the less resistant ones had been weeded out and only the more resistant remained Under such conditions there does not seem to me to be sufficient evidence available to justify the neglect of the resistance of certain strains of parasite to permanent cure as a possible cause of the chronicity of benign tertian infections among repatriated War cases Such a very selected population may have been infected entirely with such resistant strains

The ease with which a majority of fresh infections with benign tertian malaria are cured as compared with chronic ones, would support the view that such process of elimination might have taken place But such apparent

elimination can also be explained as due to a greater power of recovery in certain individuals as much as to a low power of resistance in some of the original strains of parasites In double infections with *P vivax* it has also been noted by several workers that one set of parasites may disappear from the peripheral blood much more rapidly than the other This result has been observed both under natural conditions and as the effect of treatment

Some recent work in these laboratories is of interest in this connection The numbers of parasites per c mm were determined in the peripheral blood of over 50 patients suffering from chronic benign tertian malaria The minimum number of parasites which were necessary to cause fever was found to vary within very narrow limits (3,500–4,000 per c mm) These findings suggest either (*a*) that all strains of *P vivax* have an equal clinical virulence, (*b*) or that previous treatment had eliminated patients infected with strains of a lesser vitality or (*c*) that repeated medication or prolonged growth in the human host had reduced the parasite to a condition of 'fixed virus'

(*b*) *Changes in the virulence of the same strain of parasite*

It is well known that certain bacteria lose their virulence after cultivation on artificial media and that this virulence can be re-established by passage through susceptible animals Although it is unwise to place too much reliance on the behaviour of bacteria as compared with that of protozoa, such a possibility may be considered, more especially as it has been found that the virulence of some of the other pathogenic protozoa have been enhanced by animal passage

In the case of the direct passage of *P vivax* through man, Reese and Peter (1924) and Marginescu (1930) state that they have found no increase in clinical virulence after repeated passage and Boyd (1925) records similar findings in the case of bird malaria On the other hand, Heimann (1924), MacBride and Templeton (1924) and Bunker and Kirby (1925) consider that an enhanced virulence has developed in the strains used by them Sergent and Sergent (1921) also found in bird malaria that a strain of parasite which had been attenuated by quinine medication regained its normal virulence after several passages through the animal host These observations suggest that increased rapidity of passage through the animal host cannot be neglected as a possible factor in causing changes in the clinical virulence of the malarial parasite

Engel (1918) has suggested that the climate in which the anopheline host lives may influence the toxic characters of *P vivax* It is well known that certain protozoal strains renew their vitality by conjugation, so it is possible that the sexual cycle induced by passage through the mosquito may rejuvenate or increase the vitality of the malarial parasite In the case of trypanosomes, however, Duke (1923, 1928) thinks that epidemic sleeping sickness is due rather to rapid direct mechanical passage through a series of animal hosts and that passage of the trypanosome through the tsetse fly tends to lower its virulence In this trypanosome the evidence of a sexual cycle in the insect host still requires confirmation

Gill (1928) considers that changes in the virulence of the strain of parasite, probably *P falciparum*, plays only a minor, if any, part in the genesis of the epidemics of malaria seen in the Punjab, and that the greater severity of the clinical symptoms recorded at such times is due to a larger dosage of sporozoites received by the host James (1924) believes that the strain of *P vivax*, with which he has been working, has increased in clinical virulence after repeated passage through the mosquito Rudolf (1927) also found that patients, refractory to one strain of parasite by direct blood inoculation, could be infected by the same strain after passage through the mosquito

The very different effects produced by treatment in the production of permanent cure in malaria induced by blood inoculation, as compared with those in infections transmitted by mosquito bite, would suggest also that passage through the insect host may modify considerably the cure-resistant properties of a strain of *P vivax* Yorke (1925) found that following quinine treatment the relapse rate after blood inoculation was only 2 per cent, while after mosquito infections it was 57 per cent The figures recorded by Davidson (1925) were 3 3 and 56 5 per cent respectively, and other workers have obtained similar results

The multiple infections which occurred during the War must have resulted in a very rapid passage of the parasite through a series of insect and human hosts in many cases The evidence summarized above suggests that such passages cannot be neglected as a possible factor in tending to raise the virulence of the parasite and causing the development of a more toxic or a more resistant strain of parasite than that originally inoculated

(c) *The quantum of parasites received by the human host*

It has been found in some other protozoal diseases that the type of infection produced is considerably affected by the number of parasites introduced into the host One theory propounded as an explanation of the severity of the clinical symptoms of malaria noted during the War was that, by the multiple bites of infected mosquitoes, such an enormous dosage of sporozoites was injected that severe infections developed Christophers (1911) records that in bird malaria the dosage of sporozoites seemed to have a very important influence on the severity of the infection produced in bird malaria, and similar observations have been noted by Sergent, Sergent and Catanei (1923) On the other hand Pijper and Russell (1925) found no relationship between the number of benign tertian parasites injected into patients and those present in the circulating blood James and Shute (1926) also report that they were unable to reach any definite conclusion that the duration of the incubation period, the severity of the attack or the liability to relapse was influenced by the size or frequency of the dose of sporozoites of *P vivax* injected by infected mosquitoes Gill (1928), however, suggests that the larger quantities of sporozoites injected during epidemics explained the severity of the clinical symptoms noticed at such times

Although an increased dosage of sporozoites may account for an increase in the severity of the symptoms and the difficulty of clinical cure, yet it does not afford any explanation of the cause of the chronicity of certain cases In nature, however, all infective mosquitoes would probably not have derived their sporozoites from the same source or strain of parasite, as was the case in the experimental work mentioned, so the greater the number of bites the greater the chance of the introduction of multiple strains of parasite This might account for the presence of both the more virulent and the more cure-resistant features recorded

(*d*) *Conclusions*

No certain proof has been brought forward that variations in the strain or virulence of malarial parasites exists, but the evidence in the case of *P vivax*, at least, very strongly supports such a view The combined weight of the observations quoted above goes to show that such variations cannot be neglected as a probable factor in the mechanism of either clinical or permanent cures in malaria

In many of the observations recorded above the patients who suffered from chronic benign tertian malaria acquired their infections during the War at widely distant places and only a comparatively small percentage of those originally infected developed such chronicity This would suggest that such virulence was not the only factor in influencing resistance to permanent cure, but that, as suggested by James (1926), the individual susceptibility or resistance of the host to infection may also play an important rôle in determining this state

Postcript—Major Covell in a personal communication informs me of a very interesting observation made by him in the Andaman Islands A small party of young adult Karen labourers were recruited by a contractor to undertake timber-felling operations They were brought directly from Burma and placed on a small uninhabited island, the site of the work These men stated that in their own country the severity and incidence of 'fever' was comparatively mild among them When 44 of these labourers were examined three months after arrival, the spleen rate was 66 per cent and the parasite rate 86 per cent Thirty-eight men showed parasites in their peripheral blood, of whom 19 had *P falciparum*, 6 *P vivax* and 17 *P malariæ* All the anopheline mosquitoes caught were *A ludlowi* and 97 of these were caught in a single hut These men were so prostrated with fever that work on this island had to be abandoned (Covell and Baily, 1927)

The labourers all looked well-fed and sturdy men There was nothing to suggest that the outbreak was due either to ill-nourishment or bad living conditions Several explanations can be suggested as causes of this outbreak —

(*a*) A new strain of parasite had been introduced, which seems unlikely as they were taken directly from their country to uninhabited islands

(*b*) The virulence of their own strains of parasite had been enhanced by rapid passage through *A ludlowi*

(*c*) The men were receiving an increased dosage of sporozoites

(*d*) The climatic conditions were such as to stimulate an increased susceptibility to the effects of the disease (Sinton, 1931)

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STUDIES IN MALARIA, WITH SPECIAL REFERENCE TO TREATMENT

Part XVI

THE RELATIONSHIP OF SEASON TO CURE RATE

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THAT the malarial fevers have a seasonal incidence has been known for years, and this has been remarked upon by many workers, more especially in connection with the spring rise of infections with *P vivax* and the æstivo-autumnal prevalence of *P falciparum* Most observers have considered that this seasonal incidence was chiefly connected with the seasonal prevalence of the carrier Anopheline and the temperature necessary for the development of the different species of malarial parasite in them Other workers, however, do not think that such explanations are the whole truth and suggest that some other seasonal factor influences either the host, or the malarial parasite in the host, in the causation of this distribution of the malarial fevers More recently certain observations have been made which suggest that the rate of cure, both medicinal and natural, may be influenced by seasonal conditions

Ross (1910) noted a seasonal periodicity of relapses in bird malaria and states (Ross, 1919) that 'the parasites may be influenced by season – that is, that relapses may be more frequent and more difficult to cure at certain seasons than at others' Riebold (1918) thinks that the occurrence and periodicity of relapses lies not in the parasite or man but depends upon external cosmic influences Marchoux (1918) considers that the disappearance of infections with *P falciparum* in France is due to climate, and that season may exert an important effect on these cures by influencing the humoral reactions of the host Patrick (1919) attributes the benign tertian relapses seen in the spring to 'a seasonal cyclical increase in the vitality of a hitherto dormant parasite' Plehn

(1919) remarks that latent infections reveal themselves in the spring and autumn, and thinks that the morbidity curves of malaria are affected by changes in meteorological conditions Von Neergaard (1920), in an Alpine climate at 4,000 feet, found that in chronic malaria cases, mostly benign tertian, the relapses were most frequent from December to April, but afterwards gradually diminished until October Manwell (1929) reports that in bird malaria there was a genuine seasonal variation in relapses among his cases These were most numerous from September to April and lowest in the spring and summer

Similar variations have been noted in some other diseases Wenyon (1926) states that *Babesia bigemina* inoculated into young animals was followed by a higher percentage of recoveries at some seasons than at others, while Reed (1930) states that there are well marked seasonal variations in the susceptibility of guinea-pigs to toxins

Stephens *et al* (1918), when investigating the effects of season on cure among malarial patients at Liverpool, arranged their cases according to the months in which treatment ended A similar method has been employed by us in the investigation of 1,240 cases of chronic benign tertian malaria treated with the cinchona alkaloids at Kasauli, Simla Hills, Punjab (altitude 6,000 feet) during the years 1924 to 1927 The conditions were such as to preclude chances of reinfection

The results of this work have been graphed in the following Chart in which the figures for the 589 cases reported by Stephens *et al* (1918) have been given for comparison In this graph the cure rates show a distinct seasonal variation This, however, is the reverse of that recorded by von Neergaard (1920) and Stephens *et al* (1918) and, more closely related to the results reported by other workers If the Kasauli figures be studied, it will be noted that although the number of the total cases observed was large yet only about one-fifth finished treatment during the six months from November to April For this reason differences due to disparity in numbers cannot be entirely excluded as a cause of the apparent seasonal distribution The results as recorded would, however, help to support the hypothesis that there is a distinct seasonal incidence in the morbidity rate in malaria, apart from any increased prevalence due to a more extensive carriage of infection by the mosquito

Most of the workers quoted above have remarked on the occurrence of this phenomenon without giving any very definite suggestion as to what feature of the season was possibly responsible for the variation The season of the year has such a marked influence on the external environment of man, his activities, his social habits, his clothing and his diet, that it becomes a very complicated problem to decide which factor, if any, has a predominant influence on causation Different observers have attributed to various of these factors a greater or lesser degree of influence on the process of cure or relapse in malaria

It is well known that excessive chilling or excessive exertion in hot climates may act as a provocative in the causation of relapse Although such factors may be influenced by the clothing and habits of man, these are not enough to explain the seasonal periodicity of relapse Human diet is subject to seasonal variations, and Schlesinger (1921) suggest that spring relapses are activated by the eating of fresh vegetables Although diet has a marked effect on human welfare and so may affect the resistance of the human host to malaria, yet no precise evidence is available on this point

Most of the explanations advanced to account for the reported seasonal incidence of relapses and cures have attributed these to meteorological conditions, such as temperature, relative humidity, barometric pressure, solar radiation, etc These conditions are so intimately correlated that in our present state of knowledge it seems almost impossible to separate them While some observers suggest that these factors have an influence upon the natural defences of the human host, others consider the action is directly upon the parasite in this host

The different meteorological factors suggested seem best considered under the following headings (*a*) Atmospheric temperature, (*b*) relative humidity, (*c*) barometric pressure and (*d*) sunlight and solar radiation

(*a*) *Atmospheric temperature*

Many workers have reported observations which suggest that a very distinct relationship exists between the cure or relapse rates in malaria and the degree of atmospheric temperature In considering this meteorological condition one must remember that it is very closely related to solar radiation, another physical factor which some observers believe to have an important influence on cure

Ross (1910) thinks that high external temperature encourages relapse, possibly due to a stimulation of the parasite He records that the parasitic infections in his birds in India tended to diminish in a cold climate and increase in a hot one He states, however, that Caccini did not think that excessive heat had much effect on relapses in human malaria Lenz (1917) reports that the curve of relapses in his cases of malaria followed that of the mean summer temperature and the duration of sunlight He considers this an adaptation to the flight of Anophelines Schaedel (1918) found at Mainz that the mean monthly curve of relapses among 375 patients with chronic infections (80 per cent due to *P vivax* and 20 per cent to *P falciparum*), followed exactly the curve of mean monthly temperature He, however, thinks that the relapses depended more on solar radiation or sunlight, which closely followed temperature but was influenced by the degree of cloudiness and relative humidity He also suggests that this is an adaptation of the parasite to the season when Anophelines are most prevalent

Kirschbaum (1918) thinks the cold weather checks the development of the parasite in man as well as in the mosquito and that it is reactivated by

spring warmth Bohme (1919) believes that relapses are commoner in warm climates as the direct effect of climatic conditions Anderson (1922) observed a large number of patients at Salonica and says 'many of these cases relapsed frequently during the hot season, during the cold season the relapses became less numerous or even disappeared, to commence again in the early part of the year' He thinks there is a natural tendency for benign tertian infections to become quiescent in the latter half of the year Marchoux (1918) suggests that the disappearance of *P falciparum* from France is due to the influence of the colder climate, while Reitler (1919) believes that high temperature and strong light have a provocative effect on both *P vivax* and *P falciparum*

Some of the results obtained in the work on therapeutic malaria support the view that external temperature has a relationship to relapse and cure Nicol (1927) believes from his investigations that warmth favours the development of the plasmodia in the human host Hecht-Eleda (1928) found that the incubation period of *P vivax* infections after intravenous injections rose from 4 or 5 days during the warmer months of June and July, to 7 days in the colder months from October to December James Nicol and Shute (1929) record that the transmission of *P vivax* to man by mosquito bite was more likely to fail during the winter months and in those persons who were allowed about in the cold, than during the warm months and in those who were kept indoors in bed These differences they think are due to cold assisting the natural curative processes of the body, and they consider it analogous to the more rapid cure of infections with *P falciparum* recorded in cold climates

On the other hand, Stephens *et al* (1918) record very marked differences in the cure rates of persons suffering from chronic benign tertian malaria receiving the same treatments at different seasons of the year The only meteorological factors they could find in any way correlated to the relapse rate were the variations in mean temperature and prevalence of the east wind The conclusion they drew was that 'the higher the mean daily temperature the higher the percentage of cures' but they were unable to state whether such observations were applicable to other countries which had different meteorological conditions Von Neergaard (1920) also records a higher relapse rate in this form of malaria from December to April, with a rise in cure rate in the warmer months of the year Pagnier and Schrumpff-Pierron (1925) state that in Macedonia the advent of malarial attacks 'is absolutely periodic and dependent upon outside temperature and meteorological conditions generally The onset of *P vivax* is determined by temperatures reaching about zero, and continues when the thermometer rises, so long as it does not go beyond 5°C to 10°C The *falciparum* outbreak only begins when the minimum temperature reaches between 25°C and 30°C and it lasts, in spite of the thermometer's progressive fall, until a temperature of about 5°C is again reached' These workers, therefore, recommend sending patients infected with *P vivax* to a warm country and those with *P falciparum* to a cold one to facilitate cure Acton (1921) could not discover any relationship between relapse rates and

temperature conditions in chronic benign tertian malaria, and considers that apparent variations in cure rate at different seasons are probably due to chance distribution of results

The stimulating effects of cold on the human organism have been remarked upon by many workers Marchoux (1918) also says that Martin found that the serum of animals kept outside in the winter was more active than that of animals kept in stables These observations would support the findings recorded in therapeutic malaria

The results of the Kasauli work graphed in the following Chart give curves in which the cure rate seems to vary inversely as the mean monthly temperature, i e, the relapse rate follows the curve of temperature as reported by most other observers From a study of these results, however, it will be seen that they are almost the reverse of those recorded by Stephens *et al* (1918) at Liverpool and by von Neergaard (1920) in the Alps These discrepancies make one wonder whether temperature *per se* is the sole determining factor or whether some of the other meteorological factors, which may be closely correlated to temperature, have a more predominant influence It may also be that the results are due to a combination of temperature with some other factor

(*b*) *Relative humidity*

In many temperate countries where a distinct cold season is present at some time of the year, the relative humidity may be at its maximum during that period In some tropical countries where there is a distinct rainy season this may occur during the warm weather

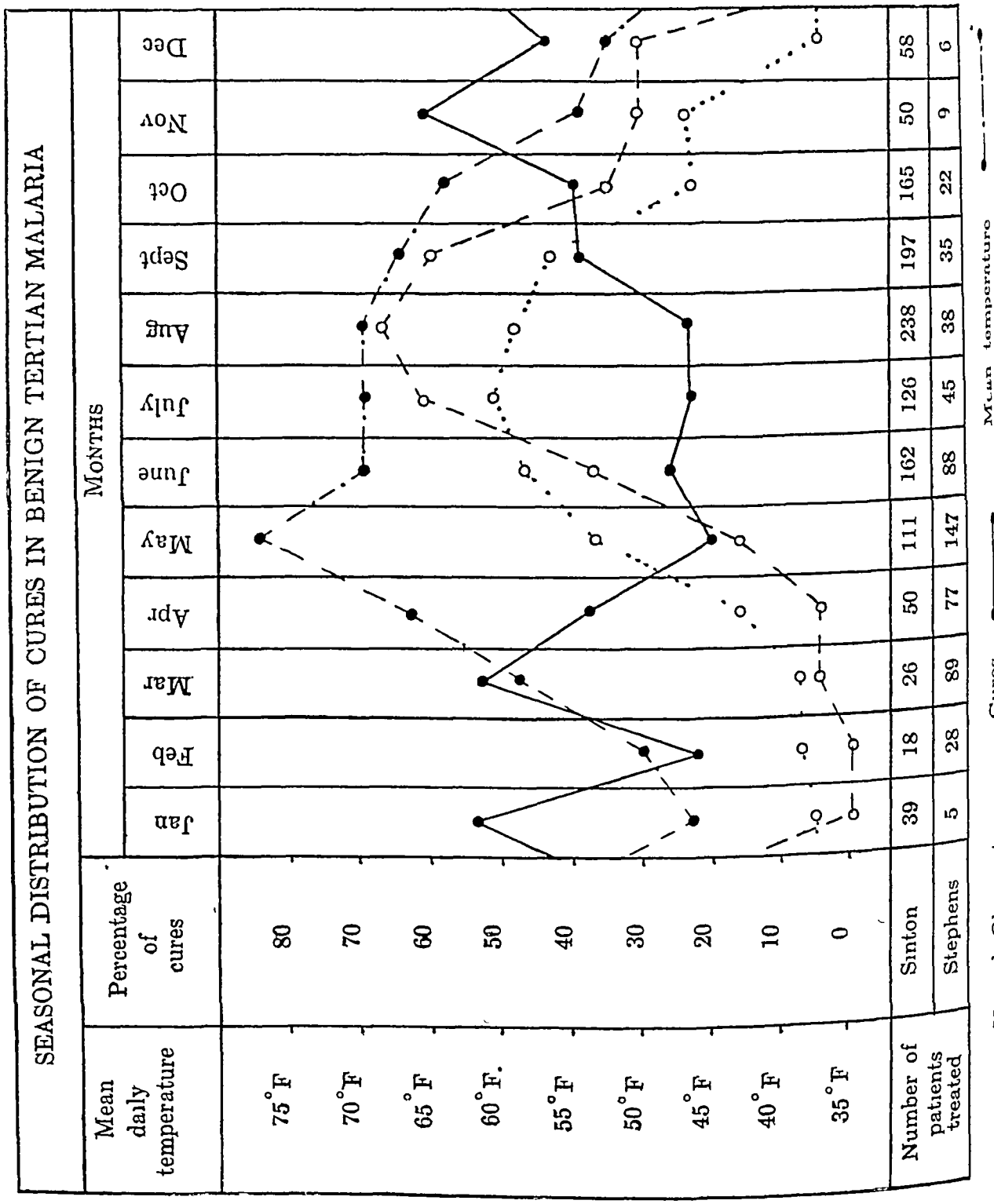
Schaedel (1918) studied the meteorological conditions at Mainz in relation to relapses His patients numbered 375 and were mainly suffering from chronic benign tertian malaria He reports that his relapse curve was at its maximum when the mean humidity was at its minimum He thinks, however, that his relapse curve was more closely related to the intensity of sunlight, which was greater when there was least humidity in the atmosphere to obstruct the sun's rays Pagniez and Schrumpff-Pierron (1925) record that their curves of relapses seemed related to relative humidity and they think that *P vivax* makes its appearance in the wet season, while *P falciparum* turns up in the dry weather in Macedonia, so long as the minimum temperature has not gone below 5°C to 10°C Manwell (1929) suggests that the high relapse rates observed by him in bird malaria during December may have been due to the association of a high temperature with a high humidity in his animal houses

Koizumi (1916), in his researches into the pathogeny of heat-stroke, found that rabbits exposed to high temperatures combined with high relative humidity, developed an increased density of the blood and a lowered alkali reserve Dorno (1924) states that sunbaths in high altitudes, in spite of the greater intensity of the sun's rays, are invigorating but in warm damp lowlands they may be enervating Buckley (1930) finds that the experience of workers on therapeutic climatology has been that high humidity is unfavourable to health

In Kasauli the relative humidity is at its lowest during April, May and the early part of June and is at its maximum during July, August and September From the following Chart it will be seen that the relapse rate rose rapidly during

CHART

SEASONAL DISTRIBUTION OF CURES IN BENIGN TERTIAN MALARIA



April, May and June and remained at an almost constant high level during July and August Thus there was little difference in the rate between the most humid and the least humid months of the year This finding does not support the hypothesis that relative humidity *per se* is of primary import in relation to cure and relapse in malaria It is possible that the combined high temperature and high humidity may have helped to keep the relapse rate high during the months of July, August and September

(c) Barometric pressure

Appel (1917) reports that the greater the disturbance of atmospheric pressure the greater the number of relapses observed in his cases He states that relapses synchronized with falls in barometric pressure Bruns (1919) and Bourcart and Laugier (1929) all think that a lowering of this pressure was responsible for sudden outbreaks of relapses in their cases On the other hand, Manwell (1929) among his birds, which were kept under conditions where such possible disturbing factors as temperature, humidity and light variations were at a minimum, was unable to determine any relationship between relapses and pressure

Ross (1910) found that when birds infected with malaria were taken to the cooler climate of the Himalayas, i e , to areas of much lower barometric pressure than Calcutta, the number of parasites in their peripheral blood became diminished Other workers have also reported that these infections tend to die out in birds taken to the hills If low barometric pressure *per se* was a cause of relapse, one would have expected an increase in the parasites under these circumstances

The Kasauli patients were treated in the Himalayan foot-hills at an altitude of 6,000 feet, so that when they were transferred there from the plains they were subjected to a fall in barometric pressure equivalent to 5 or 6 inches of mercury within a couple of days The quinine treatment of these patients was stopped when they were sent to Kasauli and no specific treatment given until a parasitic relapse occurred (Sinton, 1926) Among these chronic benign tertian patients about 23 per cent of the observed relapses occurred during the first week after arrival, 26 per cent in the second and 14 per cent in the third If these are compared with patients who had been in Kasauli for some months and had finished a course of treatment with one of the cinchona alkaloids, it is found that of the observed relapses in the latter cases, 5 per cent occurred during the first week after finishing treatment, 29 per cent in the second and 27 per cent in the third This higher percentage of relapses during the second and third weeks was also recorded by Acton (1921) among his cases in the adjacent hill-station of Dagshai These results would suggest that the sudden change in barometric pressure was a stimulus to relapse One must remember, however, that these patients had also been subjected to a considerable change in atmospheric temperature in coming from the hot plains to the cool hills This change might also account for the increased relapse rate during the first

two weeks after arrival The changes in barometric pressure experienced were much greater than any to which patients would normally be subjected, except by change in altitude These changes threw a great additional strain upon the circulatory and respiratory systems of the patient, which may have helped to stimulate relapse

A considerable number of latent primary infections were detected at Kasauli several months after the subjects had arrived there, while one would have expected them to occur at an earlier date if barometric changes had a powerful influence

Unfortunately no systematic records of the barometric pressure at Kasauli are available, but the monthly means of the adjacent hill-station of Simla have been taken for comparison A low barometric pressure seems to correspond to a high relapse rate, but the period of low pressure occurs during the monsoon months from the middle of June to the middle of September and this is also a period of high temperature and relative humidity with little direct sunlight The results recorded by Manwell (1929) with bird malaria, quoted previously, were obtained under conditions where there was no marked variations in these factors This suggests that any apparent correlation of barometric pressure with relapse and cure rates is probably mainly due to the effect of changes in this factor on temperature and humidity rather than a true correlation

From the results quoted above, the conclusions arrived at were that there is not sufficient evidence to implicate the degree of lowering of barometric pressure normally experienced by patients as a cause of relapse It rather suggests that the closely related factors of temperature and humidity may be the cause of any apparent correlation

(d) Sunlight and solar radiation

The intensity of the sunlight and the solar radiation are so closely connected with atmospheric temperature, relative humidity, cloudiness, altitude, etc, that it is very difficult to evaluate the effects due to each of these causes separately Many observers for this reason have considered that the seasonal incidence of relapses is correlated rather to solar radiation than to temperature *per se* Apart from its meteorological relationships, solar radiation is composed of many different spectral rays which, both quantitatively and qualitatively, may vary very greatly in their action on the human organism The relative intensity of these rays in solar radiation differs markedly at different times of the year, in different latitudes, at different altitudes and under different meteorological conditions Until our knowledge of the elements concerned in the effects of solar radiation are more clearly understood, it seems impossible to judge which factors, if any, are chiefly concerned in the causation of the reported seasonal incidence of malarial relapses

A very great amount of research has been done in recent years on the effects of solar radiation on the human organism, but it is impossible in an article of this nature to enter into a discussion of this work A very few of

the findings which seem more directly related to the subject under discussion will be quoted as occasion arises

Exposure to sunlight has been found to cause an increased activity of the leucocytes (Colebrook, 1924) and a change in the leucocytic formula (Taylor, 1919) Gauvain (1930) consider that 'the benefits of insolation are due not so much to the intensity of light as to shock of varying stimuli eliciting a favourable response It has long been known that the morning sun has the greatest therapeutic value, yet it is less intense than the light of the midday sun This variation in response is due to the fact that the light shock evokes a greater response immediately following antecedent darkness Thus paradoxically as it might appear, darkness is as essential to heliotherapy as light Continuous exposure to sunlight in summer would not produce beneficial results as speedily as alternations of light and shade, heat and cold, humid and dry air' There is some evidence to suggest that excessive exposure to very strong sunlight may tend to have a detrimental and not a beneficial effect on health

Several workers have reported a correlation between the period of maximum sunlight and that of maximum relapses in malaria Lenz (1917) at Punchheim and Schaedel (1918) at Mainz think that the period of maximum sunlight is that of maximum relapse and that it acts by stimulating the gametocytes to parthenogenesis Kisskalt (1917) in Prussia reports that the maximum relapse rate in chronic benign tertian malaria was from April to July and he attributes the spring relapses to the effects of sunlight* Von Heinrich (1917) at Sarajevo noted that in winter the intervals between relapses were long and that these were slight in character, whereas their frequency and severity increased in the spring* These differences he thinks were due to the action of sunlight and he states that he succeeded in producing relapses in some benign tertian infections by exposing his patients to direct sunlight for an hour Reitler (1919) at Vienna found that the number of positive blood findings rose from a minimum among benign tertian cases in February to a maximum in May This he considers due to the provocative influence of higher external temperature and stronger light acting on the parasites Martini (1921), from a study of relapses in Macedonia, came to the conclusion that relapses in benign tertian malaria were provoked by sunlight, which acted on both the human host and the parasite, and therefore relapses were commoner in light-skinned than dark-skinned persons Hewetson (1922) attributes an unusual outbreak of black-water fever to an exceptionally dry and sunny summer in Rhodesia He thinks sunlight may have an intimate relationship with the health of the red blood cells Kligler and Weitzman (1926) found that exposure to sunlight produced a lowered resistance to trypanosome infections in animals, and suggest that this might also be a factor in malarial relapses

*Dorno (1924) states that the abundance of strongly penetrating ultra-red rays found in the spring sun furnishes an explanation for the frequently observed fatigue on exposure to the sun's rays in the spring

Kunsloft (1928) believes that the relapses are not due to direct sunlight but rather to the intensity of the light which modifies certain properties in the blood Sellards (1918) found that direct sunlight did not affect the malarial parasite in defibrinated blood *in vitro* Di Pace (1923) reports that solar radiation had little value as a provocative of malarial relapses and Manwell (1929) was unable to cause relapse in bird malaria by exposure to sunlight The work of Boyd (1929) on the effect of light on the parasite cycle in bird malaria is interesting This worker has found that the segmentation time could be changed by keeping infected birds in the dark during the day and in light during the night

If the Kasauli figures given in the Chart are studied it is seen that cure rate fell steadily from March to reach a minimum in May During these three months the sunlight is very bright and increased in intensity, while the cloudy days are very few This would support the view that sunlight is a provocative of relapse in malaria Against such a view is the fact that during July and August, the cloudiest months of the year, when there was little direct sunlight, the relapse rate remained very high, while during October and November the brightest months of the year the rate was almost at its minimum These facts are contrary to the hypothesis that the relapse rate is directly proportional to the amount of the sunlight

The diversity of the results recorded above may possibly be due to the different effects of different intensities of sunlight In temperature climates the curve of sunlight apparently follows the curve of temperature closely, but this may not be the case in the tropics In Kasauli during the months from April to September the sunlight, when present, is intense and may exert a provocative action during April, May and early June before the onset of the monsoon During the monsoon period, from the middle of June to the middle of September, however, the sunlight is continually cut off by heavy clouds It is possible that the relatively high temperature and high humidity during these months may prevent a recuperation of the body from the depressing effects of the strong sunlight experienced during the preceding months If the idea of Gauvain (1930) is correct, that alternation of conditions is more beneficial, this would account for the higher cure rate during the six colder months of the year, from October to April During these months the degree of cloudiness is only half that seen during the warmer months, the sunlight is less intense, there are greater differences between the maximum and minimum temperatures, and there is a longer period of darkness alternating daily with light During the months from April to September the sunlight, when present, may be intense, while during the monsoon period when the amount of direct sunlight is small the depressing combination of relatively high temperature and high humidity is present

Sunlight is made up of so many different spectral rays, which vary so markedly in intensity under different conditions, that it is possible that the diverse results obtained may be due to variations in the amounts of some of these rays at different times of the year Some observers, while believing that

sunlight has an effect in the causation of relapses, go even further and suggest that this action may be due to certain of the constituent rays, chiefly the ultra-violet

Finsen has shown that ultra-violet energy is absorbed directly into the blood Colebrook, Eidinow and Hill (1924) found that exposure to these rays caused an increase in the activity of the leucocytes, while Irala (1920) records that over-exposure may eventually damage these cells and arrest phagocytosis De Groer (1922) reports that these rays cause first a leucopenia and then a leucocytosis He considers that their action is in the nature of a protein shock Levy (1916, 1919) and Gassul (1919) found that in mice and rats it was possible by means of these rays to cause marked enlargement, hyperæmia and hæmorrhagic changes in the spleen and other organs, such as the liver, lungs, and kidneys Such effects might have a considerable action on malarial infections

The effects of these rays have been tried by several workers on malarial patients May (1918), Moreau (1918), Reinhard (1919) and Sassen (1919) report successful results with these rays as a provocative of relapse in malaria Whitmore (1922) has reviewed the literature on the subject of ultra-violet rays and sunlight in relation to relapse in malaria He found that in bird malaria exposure to these rays caused relapses and also increased the number of parasites in the peripheral blood Several workers have attributed the spring relapses in malaria to the influence of these rays

The absence of any precise data on the intensity of these rays in the different observations makes it impossible to evaluate the rôle of this factor in relation to relapse and cure rate in malaria, but there seems some evidence that it may have a distinct influence

The statement of Dorno (1924) that the sun's rays have a higher ultra-red radiation in spring and a higher ultra-violet in the autumn suggests that the action of the former rays requires investigation, more especially as these rays are said to have a fatigueing effect

Another factor in the action of light which has been suggested by some workers is that it increases the effect of quinine medication It is known that fluorescin or eosin in the presence of light will dissolve red blood cells *in vitro*, but there is no such action when the experiment is carried out in the dark Fluorescin itself has not been found to have any curative action in malaria, but it has been suggested that the action of quinine might be dependent in part upon its fluorescent properties

Sellards (1918) found *in vitro* that quinine had no hæmolysing action of the red cells in the presence of sunlight An Editorial in the *Journal of Laboratory and Clinical Medicine* (1927) states that Bass observed that malarial parasites will grow in the presence of quinine in the dark and not in the light It was, therefore, suggested that light may be a factor in the parasiticidal action of quinine Ruszny ak (1920) reports that the photodynamic action of a mixture of quinine and eosin *in vitro* was greater in proportion than

that produced by either separately These findings suggested to him that better results in malarial treatment might be obtained by a combination of these two drugs He records that the results of experimental treatment supported his hypothesis Votaw (1926) also thinks that a combination of these two substances was beneficial in chronic malarial cases Viale (1920) states that the action of quinine in rebellious cases of malaria was enhanced by exposing the nude body to the sun for several hours after each dose of the drug

In Kasauli the patients who finished treatment during May, June and July were under quinine treatment during some of the brightest months of the year, yet the cure rate was at a minimum in these cases These months are also those in which the patients were in their thinnest clothes and were, therefore, most likely to receive the maximum effects of sunlight, as compared with the colder winter months These results do not support the hypothesis that sunlight *per se* aids the action of quinine through an increase in its parasiticidal power

While dealing with light it is interesting to record that various workers have tried X-rays as a means of facilitating cure in malaria and also as a provocative of relapses The general consensus of opinion seems to be that, while small doses may be beneficial, large ones are harmful (Pais, 1919, 1919*a*, 1923, Cordier, 1920, Cignolini, 1923, Pigt, 1924, Heymann, 1925) Padilla (1926) considers those rays to be one of the best provocative agents, while Pais (1919) thinks such radiation increases the efficacy of quinine The latter author (Pais, 1919) thinks these rays act by stimulating the natural defences of the body and not by any action on the parasites

Conclusions

As the result of our investigations the tentative conclusions arrived at were that —

(*a*) There appears to be a seasonal incidence in the relapses of benign tertian malaria, at least, and the results of other workers suggest that this may also occur in the other forms of malaria

(*b*) The exact nature of the factor responsible for these variations in the mechanism of cure and relapse is still doubtful

(*c*) There is considerable evidence to suggest that the relapse rates may be correlated to atmospheric temperature

(*d*) It does not seem probable that relative humidity *per se* influences the relapse rate, but it is possible that a high relative humidity may have an effect when combined with high atmospheric temperature

(*e*) No evidence was found that the variations of barometric pressure to which patients are ordinarily subjected, have a marked influence on relapse It is suggested that any apparent correlation may be due to changes in the other meteorological factors, which may accompany pressure variations

(*f*) The Kasauli results do not support the hypothesis that relapse rate is proportionate to the amount of direct sunlight It seems possible, however,

that the very intense sunlight during warmer months may have a deleterious action as compared with the sunlight of the winter months

(*g*) Further investigation is required to determine the relative effects, if any, of the different solar rays on the mechanism of cure and relapse

(*h*) The evidence supports the view that the meteorological conditions present in Kasauli during the colder months of the year may have a favourable effect on the mechanism of cure

(*g*) The results emphasize the necessity for control treatments carried out at the same time as tests are made of the value of any medicinal measures (Sinton, 1926)

(*h*) In our present state of knowledge season cannot be neglected as a factor which probably influences the mechanism of cure and relapse

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THE NUMERICAL PREVALENCE OF PARASITES IN RELATION TO FEVER IN CHRONIC BENIGN TERTIAN MALARIA

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ALTHOUGH many workers have made relative estimations of the parasite prevalence in the peripheral blood in different stages of malarial infections, yet few investigators have made precise counts These relative estimations have been based mainly upon such determinations as the average number of parasites per microscopical field or in relation to the number of blood cells Such methods, while giving a rough approximation to the relative numbers, are necessarily subject to a considerable degree of error The necessity for more precise methods of investigation of the malarial fevers has been apparent for some time and with this object in view more exact parasite counts have been made in a number of chronic benign tertian infections

A large number of patients suffering from chronic infections with *P vivax* are sent annually to the Malaria Treatment Centre at Kasauli for special treatment, and it was from this population that our cases were drawn These patients are young adult British soldiers, whose histories are that they had relapsed on many occasions after the termination of various courses of quinine treatment The blood of these patients is examined by the thick-film method on arrival at Kasauli and weekly thereafter until a parasitic relapse is detected During this diagnostic period no malarial treatment is given In these weekly examinations it is not uncommon to detect parasites before any pyrexial symptoms have appeared, so it was possible to make estimations in the pre-pyrexial period of relapse Unfortunately, as these patients are soldiers, treatment had to be commenced at a very early date, so it was not possible to follow the cases over long periods without the disturbing effects of medication

TECHNIQUE

In the past various methods have been used in attempts to ascertain the exact numerical prevalence of malarial parasites in the peripheral blood at

different stages of the disease One of the earliest methods was by the use of the Thoma-Zeiss haemocytometer, but with this technique small parasites are very liable to be overlooked This was soon replaced by a method which has had a more extensive use In this the comparative relationship between the number of parasites and the number of blood cells, either red or white, was determined and from the results of a blood count, the absolute number of parasites per c mm calculated This method is, however, cumbersome and the margin of error is large Thomson (1911) published a more accurate method in which the numbers of parasites were counted in a measured quantity of blood and the same principle was used in a technique for field work introduced by Christophers (1921) This system is more accurate than any of the other methods previously described but, unfortunately, requires very considerable technical skill and preparation for its use Since the 'fowl-cell' method was devised by Sinton (1921), it has been extensively used in India and the older methods have been discarded This method has been found eminently practical and reliable for both field and clinical work and is that used in the investigations recorded below

The number of parasites per c mm of peripheral blood was determined in over 50 cases of chronic infection with *P vivax* In making the counts the parasites were enumerated in, at least, 1/20th of a cubic millimetre of blood, and in the majority of cases twice this amount was examined It was usually only possible to do one count before treatment was commenced, so no continuous records of fluctuations of the numbers of parasites in individual patients at different stages of the disease, are available

Results of the investigation

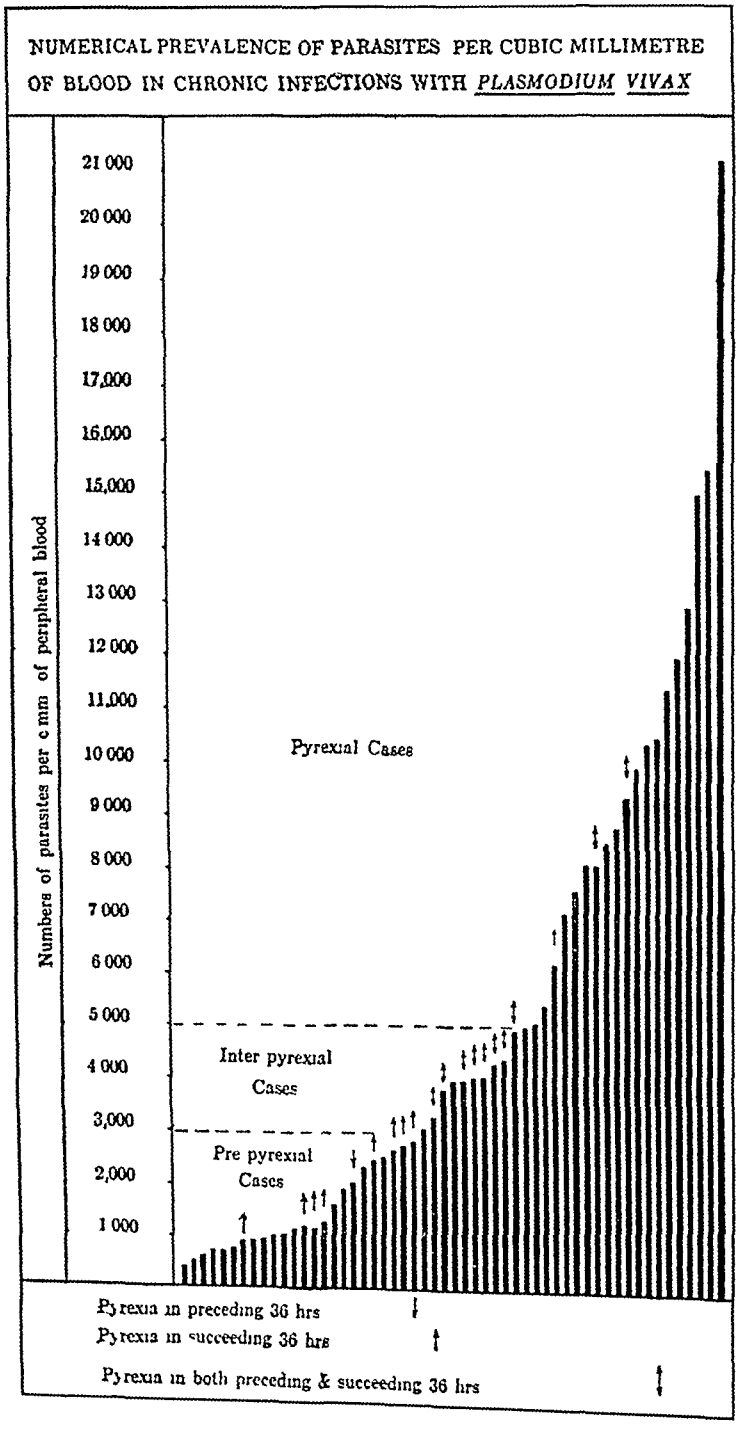
As our routine diagnostic examinations of the blood were made weekly, a number of parasitic relapses were detected before the occurrence of any febrile manifestations Twenty-six infections were studied at this stage, of which all except two showed counts between 336 and 3,000 parasites per c mm The exceptions had counts of 3,960 and 6,160 and these patients may possibly have had undetected febrile symptoms before the enumerations were made The average count in these pre-pyrexial cases was 1,680 per c mm

Eleven patients were examined in the apyrexial interval following a febrile paroxysm and the counts in eight of these lay between 3,000 and 5,000 per c mm One low count (1,960) followed a pyrexia of only 99°F, while the other two were 8,000 and 9,240 respectively

Seventeen counts were made on patients with pyrexia at the time the blood was taken The majority of these were in the defervescent stage of the paroxysm, so sufficient time had elapsed in the majority of instances for segmentation to have taken place more or less completely All the counts lay between 4,880 and 21,120 per c mm with an average of 10,160 per c mm

These results have been shown graphically in the following Chart, from which it can be seen that the counts fall almost exactly into 3 main groups —

CHART



(*a*) *Counts of* 3,000 *per c mm or less*.—This group contains 25 counts, all made on patients who showed no pyrexia at the time. Of the 26 patient in whom blood parasites were detected before pyrexial symptoms were recognized, 21 are included in this group. For this reason the group has been called the 'pre-pyrexial'.

(*b*) *Counts between* 3,000 *and* 5,000 *per c mm*.—There are 10 counts in this group and these were all made on patients of whom only one showed pyrexia (count 4,880 per c mm.). This group includes 8 out of the eleven counts done in the apyrexial interval following the first recorded febrile paroxysm of the relapse. This group has been called the 'inter-pyrexial'.

(*c*) *Counts of* 5,000 *and over*.—This group of 19 cases includes all the 17 counts made during pyrexia except one of 4,880. This group is called the 'pyrexial'.

This method of grouping seems a very suitable one for use in future work.

Discussion of results

Various estimations as to the number of parasites needed to produce fever in malaria have been made, thus Sims (1902) thought that 400 parasites per c mm. would cause fever, and Ross (1911) estimated that at least 50 parasites per c mm. were needed to produce pyrexia. Ross and Thomson (1910) carried out a series of exact enumerations of parasites in the peripheral blood of eight patients suffering from infections with *P. vivax*. They report that with this species as many as 1,500, 852 and 540 parasites per c mm. might be found without fever and as few as 150 and 50 per c mm. in cases with very slight fever. They concluded 'that about 200 to 500 may perhaps be taken as the usual limit' required to produce fever. Unfortunately many of the counts were made when the patients were under the influence of quinine.

Several workers have investigated the relative parasite prevalence in infections with *P. vivax* artificially induced for therapeutic purposes. Korteweg (1924) found that the 'initial fever' might commence when the number of parasites was as low as 1 to 20 per c mm., but in cases who had previously suffered from malaria, the figure more closely approximated that found by Ross and Thomson (1910). In the later and real malarial rises of fever he estimated the parasites to vary from 7 to 4,500 per c mm. during high pyrexia. Many observers have found that the febrile symptoms, in artificially-induced malaria due to *P. vivax*, may commence even before parasites can be detected in the peripheral blood. Thus Grant and Silverston (1929) state that the commencement of the fever generally preceded the appearance of parasites by one to four days. It often coincided with this while occasionally it forestalled every sign of fever by two to four days.

These figures would indicate that, in fresh infections with *P. vivax*, the number of parasites in the peripheral blood may be very few or even undetectable during the primary febrile attack in some instances, while in persons who

had previously suffered from this infection a greater number is required to cause fever

The pyrogenic threshold

Most of the estimations of the pyrogenic limit have been based upon counts of the number of parasites present in the peripheral blood at the time of pyrexia, that is after schizogony has commenced These counts must, therefore, be based largely on the numbers of post-segmentation forms which appear in the peripheral blood at that time It is known that the pyrexia depends on the bursting of the mature schizonts The number of these would, therefore, be a truer guide to the fever threshold than the number of young forms derived from them which appear in the peripheral blood The latter are liable to be destroyed by many agencies soon after liberation and, as will be noted later, this destruction is probably as high as 60 per cent in some instances It is, however, difficult to judge the exact moment of schizogony and, even when this is known, it seems probable that blood cells infested with mature parasites are more liable to accumulate in the internal organs than cells with young forms As Manson very aptly said 'the forms seen in the peripheral blood are but a part of the great drama that is being enacted in the spleen and other internal organs' In practice, however, the period of pyrexia seems to be the most suitable time for counts to determine the pyrogenic limit, more especially if these can be correlated to counts made in the preceding apyrexial interval

Counts made during the first paroxysm of fever in a patient, receiving no treatment and who had previously been free from pyrexia, give us an estimate of the number of parasites correlated to the onset of pyrexia Parasite counts made during attacks of fever on the following days only indicate that a certain number of parasites may cause fever, but do not show the pyrogenic threshold in the individual under observation In the same way the parasite counts in these succeeding paroxysms may be influenced by the presence or absence of any acquired immunity, more especially in chronic infections Thus the results of Ross and Thomson (1910) and of Bohm (1918) suggest that in some cases there may be a decline in parasite prevalence in succeeding paroxysms It is also well known that if patients suffering from chronic infections with *P vivax* are merely put to bed without any specific treatment, the parasites tend to disappear from the peripheral blood A similar decline has been observed in the case of bird malaria at the later stages of infection Counts made in attacks of fever occurring during quinine or similar medication afford no true index of the numbers of parasites required to produce fever Experimental evidence goes to show that the action of quinine is most marked in the case of young forms of parasite It is, therefore, probable that while the number of segmenting forms is sufficient to cause fever, yet the drug may have destroyed so many merozoites that the number found in the peripheral blood, at a later date, is no index of the original prevalence of the mature parent

schizonts For this reason the results of Ross and Thomson (1910), which are based on counts carried out both before quinine treatment had been started and while it was being given, are not strictly comparable with our results, which were obtained before the commencement of treatment

If the protocols of Ross and Thomson's experiments are examined it is possible to collect from them 27 counts in which the influence of treatment can be neglected Of 11 counts made during apyrexia only one is higher than 228 per c mm, while in the pyrexial cases the lowest counts were 284, 308 and 370 per c mm The data given about these cases suggests that they were not chronic infections, so that between 200 and 500 may perhaps be taken as the usual pyrogenic limit in such cases

In our patients (*vide* Chart) there seems to be a very definite pyrexial threshold at 5,000 parasites per c mm This represents the survivors of the act of schizogony and not the number of the parent schizonts which were responsible for the pyrexia

If the apyrexial group is examined it will be seen that only one of the eleven counts of less than 1,000 per c mm has a recorded pyrexia within the succeeding 36 hours Of the seven counts between 1,000 and 2,000 three developed fever, and of the seven between 2,000 and 3,000 in four febrile symptoms were recorded The counts in the cases which developed fever at the next segmentation suggest that the number of mature schizonts necessary to cause pyrexia lies between 1,000 and 3,000 per c mm in chronic infections with *P vivax*, and is probably nearer the former limit than the latter

A higher fever threshold has been observed in our chronic infections as compared with that recorded by other workers in fresh infections This supports the view that a tolerance to the clinical effects of the infection has been developed in the former infections

In our chronic benign tertian infections there seems to be a very definite pyrogenic threshold equivalent to about 5,000 young parasites per c mm in the peripheral blood The apyrexial interval between the initial paroxysms were characterized by counts between 3,000 and 4,000, while in the pre-pyrexial periods the counts were less than 3,000

The clearly defined character of these findings suggest several explanations (*a*) that *P vivax* has a very definite pyrogenic threshold or (*b*) that the patients who suffer from chronic infections have a very similar degree of susceptibility to the 'toxin' of *P vivax*, or (*c*) that previous treatment had eliminated all strains of parasite of different virulence, or (*d*) that continued courses of treatment or prolonged sojourn in the human host had reduced the parasite to a condition of 'fixed virus'

The work of other investigators on fresh infections indicates that the number of parasites necessary to produce fever may vary very markedly This may be due either to differences in the virulence of the strain of parasite or in the susceptibility of the human host Rudolf (1924), Pijper and Russell (1925), Bunker and Kirby (1925), and Lilly (1925) have all recorded marked

differences in the clinical effects of different strains of *P vivax* used by them in therapeutic malaria These findings would negative the view that normally there is a constant pyrogenic threshold for all strains of this parasite

James (1926) and other workers have produced evidence to show that different individuals vary markedly in their degree of susceptibility to the clinical effects of malarial infection and also in their resistance to permanent cure A very feasible explanation of our results would, therefore, be that continued courses of treatment had eliminated all the more resistant and normally susceptible individuals and left a population of persons with almost a uniform resistance It is also possible that the previous quinine treatment had in time eliminated strains of parasite of varying degree of toxicity and left only a special resistant strain The hypothesis of a 'fixed virus' would also explain the results In our present state of knowledge of the virulence of the strains of parasite and of the individual immunity of the host, it is impossible to decide which of these views is the most probable It, however, suggests that much light would be shed on the mechanism of malarial infection and cure by researches along these lines

The relationship between the number of parasites and the degree of fever

Ross and Thomson (1910) found a relationship between the number of parasites and the degree of pyrexia in malarial patients, while Rudolf and Ramsay (1927) record a similar relationship in 5 out of 10 cases of artificially-induced infections with *P vivax* On the other hand Bohm (1918) in natural malaria and Pijper and Russell (1925) in therapeutic malaria could trace no such relationship

Ross and Thomson (1910) point out that 'it is probable also though by no means certain, that the resistance to the toxins of the *Plasmodia* varies, not only in different persons but in the same person at different stages in the course of his infection and under different physiological conditions' The recent extensive work with artificially-produced malaria amply confirms this hypothesis Under such conditions one would not expect the same number of parasites to cause the same degree of pyrexia in all individuals, but that there might be a distinct correlation between these in the same individual under the same conditions

Unfortunately two- or four-hourly records were not kept of temperature so the maximum degree of pyrexia is not available in our cases The highest temperatures recorded seemed to occur chiefly with the high parasite counts

Relationship of parasites to fever

Ross and Thomson (1910) have compared the numbers of parasites counted on days with fever with those counted on days without fever A febrile day was taken as any one on which the patient's temperature exceeds 98 6°F , or any one which comes between two tertian paroxysms They found that the average parasite counts on febrile days to be 4,535 and on afebrile days 125 Unfortunately the individual counts had in many instances been

influenced by quinine To obviate this fallacy, we have taken from their protocols such counts as would appear to have been done at times when treatment had no influence Of 16 pyrexial counts varying from 284 to 28,700 per c mm the average was 5,580, while of 11 afebrile counts varying from 28 to 852 per c mm the average was 184 Using this method of division into febrile and afebrile groups our results show 25 afebrile counts varying from 336 to 3,000 with an average of 1,420, and 29 febrile counts varying from 3,235 to 21,120 with an average of 8,012 per count

Rudolf and Ramsay (1927) also found that 'as the temperature became greater the parasites increased, and decreased as the rises of temperature became smaller' These results and the figures on the Chart show that there is a distinct relationship between the number of parasites and the occurrence of fever The higher average level of parasites on the febrile days in our patients as compared with those of Ross and Thomson (1910) supports the view that patients with chronic infections have developed a greater tolerance to the clinical effects of parasite infestation than those suffering from fresh infections

Increase in the number of parasites during a paroxysm

P vivax is usually believed to produce from 15 to 20 merozoites at each schizogony, so theoretically the parasite count should be 15 to 20 times as great after a paroxysm than before one It is, however, well known that the rate of increase of malarial parasites in the peripheral blood usually falls far short of that which would be expected if each merozoite produced at schizogony survived

Taliaferro (1925) found in bird malaria that about 66 per cent of the estimated number of merozoites produced at segmentation did not reach maturity The evidence produced by this worker indicates that in bird malaria the number of parasites in a fresh infection goes on increasing until a maximum is reached (or death occurs), after which there is a more or less rapid fall In the early stages of fresh infections, it would seem that the natural immunity of the animal is capable of destroying numbers of parasites, but the rate of increase is greater than the rate of destruction, and therefore the parasite count rises When, however, an acquired immunity has developed the rate of destruction exceeds the rate of production and the parasite count drops

Bohm (1918) records a case of benign tertian malaria in which the parasites were 87,500 per c mm during fever, 11,000 during the afebrile period and 41,100 during the next attack, i e, an increase of 3 7 times Pijper and Russell (1925), in artificially-induced malaria due to *P vivax*, found that in seven cases the increase varied from 3 to 14 times Rudolf and Ramsay (1927) record that in therapeutic malaria 'there is a very high percentage of instances where the young forms are not even ten times as numerous as the forms from which they originate'

The results of counts made before and during the paroxysms are available in three of our cases and were as follows —

Pre-pyrexial period	Pyrexial period	Increase
2,680	11,900	× 4 4
4,000	15,340	× 3 8
4,320	8,628	× 2 0

The number of patients examined is too few to draw any definite conclusions but the results suggest that in patients suffering from chronic benign tertian infections the power of destruction of parasites at the time of schizogony is greater than in fresh infections One would expect this, as it seems almost certain that such chronic infections must have developed some degree of acquired immunity greater than that present in fresh infections, even although this was not sufficient to produce a permanent cure

The fact that in our results the counts made in the inter-pyrexial intervals are mostly lower than those made at the time of pyrexia also suggests that a considerable destruction of parasites is taking place at the latter time

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HÆMOLYSIS BY AMINES AND ALLIED SUBSTANCES

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IN previous papers (Sen, Roy and Mitra, 1929*a*) it has been shown that the effect of acid and alkali in hæmolysis is dependent largely on the concentration of H° and H′ ions and that alkali has a peculiar action of retarding or accelerating the hæmolytic behaviour of certain chemical hæmolytes according as it is added together with or after the chemical hæmolyte It has also been stated by us in a note in *Nature* (Sen, Roy and Mitra, 1929*b*) that the behaviour of serum is almost analogous to the behaviour of alkali and it has been suggested that the peculiar action of the serum may be due, at least to some extent, to the alkali content of the serum In order to throw more light on this retardation and acceleration phenomenon in presence of alkali, some further work was started with very low concentrations of ammonia, amines and allied substances It was, however, soon found that detailed work with these substances would be desirable from several points of view, and the present paper gives some results of this investigation

HÆMOLYSIS BY AMINES

Only stray references are obtainable in the literature about the hæmolytic action of amines, and most of them are not relevant to this investigation Hermann Fuhner and Earnest Neubauer (1907) experimented on the hæmolytic behaviour of some amines in homologous series and a parallelism was found between their hæmolytic action and their physico-chemical properties, they also found that the action depends on the concentration of OH′ and H° ions in the solutions used With regard to the effect of OH′ ions, Oscar Gross (1910) observed the hæmolysis of low concentrations of red blood corpuscles

by ammonia, sodium hydroxide, and sodium carbonate and found that the relationship between the concentration of the ammonia and the carbonate, and the time necessary for complete hæmolysis can be represented by the equation $C^m T = K$, where C = concentration, T = time of hæmolysis, K = constant and the value of m varies between 0 65 and 9 71 The ammonia or carbonate appeared to be adsorbed by the corpuscles, and the amount adsorbed regulated the rate of hæmolysis It was also observed that this relationship only holds so long as the amount of ammonia present is large compared with that used up in hæmolysis or by-reactions When small quantities of ammonia are employed, so that the time necessary for complete hæmolysis is large, nearly all the ammonia is adsorbed, and the rate of hæmolysis is proportional to the amount of ammonia and inversely proportional to the concentration of the blood corpuscles In the case of sodium hydroxide it was found that the reaction equation is $C^{1\cdot 3} T = K$ Some more data summarized by Hober will be given later on

A question of considerable importance to the study of the kinetics of hæmolysis has recently been raised by Christopher's (1929*a*) Hitherto it has been thought that the time-dilution curves of hæmolysis give a true representation of the action taking place in a mixture of corpuscles and hæmolyte, but according to Christopher's, the observed hæmolysis is the result of processes taking place within the cell and having nothing to do with the reaction in the mixture, and as such the time-dilution curve has not much significance The present writers, however, feel that a different interpretation can be given to the results obtained by Christopher's and this will be attempted in a future paper It should be pointed out here that the results presented in this paper were obtained before the publication of Christopher's' paper, and the plan of presenting the data has been kept almost similar to that of our earlier paper

Experimental conditions

The present work deals with the hæmolytic behaviour of methylamine, ethylamine, diethylamine, piperidine, ammonia and caustic potash, all either alone, or sometimes in presence of normal serum Experiments have also been made on the effects of ammonia, ethylamine and piperidine of equal concentrations in the hæmolysis by oleate and taurocholate, the ammonia and amines having been added both together with and after the addition of the hæmolyte to the corpuscles Solutions of different hæmolytic substances were prepared in normal saline The amines were obtained from Merck, and the solutions were standardized against hydrochloric acid In each case one c c of corpuscles of known concentration was used, the total volume of the reaction mixture being 5 c c In Tables I to VI, the time-dilution results with different hæmolytes and different concentrations of the corpuscles are given The corpuscle concentrations are shown according to Christophers' method Thus 10 grammes per litre means that 1 0 c c of 5 per cent suspension has been used in a total volume of 5 c c

TABLE I

Hæmolyte—Methylamine

Concentration of methyl-amine in moles/litre	Time in minutes of complete hæmolysis of corpuscles of concentration per litre of the mixture			
	10	5·0	2·0	1·0
0·003			65 25	
0·006			31 45	27 31
0·009	54 53	30 36	21 00	19·95
0·012	37 93	24 03	16 16	15 03
0·015	27·20	16 20	11·68	10·86
0·018	20·26	11 96	10·00	9·28
0·021	16·33	9·88	7·71	7·28
0·24	12 90	8·6		
0·027	10 20	7 03		
0·030	8·03	6·25		
0·033	6·13	5 43		
0·036	5 10	4 65		

TABLE II

Hæmolyte—Ethylamine

Concentration of ethylamine in moles/litre	Time in minutes of complete hæmolysis of corpuscles of concentration per litre of the mixture			
	10	5 0	2 0	1 0
0·003			57 70	38·53
0 006	48·25		28·08	21 21
0·009	28·03	19·40	17·81	13·90
0·012	15 70	13·75	11 23	8·10
0·015	11 41	8·70	7·63	6·05
0·018	8·61	6·08	5 75	4 40
0·021	5 83	4 50	4 46	0·50
0·024	4 33	3·66	3·50	
0·027	3·38	2·80	2·73	
0·030	2·83	2·25	2·16	
0·033	2·41			
0·036	2·05			

TABLE III

Hæmolyte—Diethylamine

Concentration of diethyl-amine in moles/litre	Time of hæmolysis in minutes, corpuscle concentration per litre			
	10	5·0	2·0	1·0
0·004	97·15			
0·008	38·60		17·93	11·66
0·010	.	16·66	12·56	7·08
0·012	21·05	12·60	9·66	5·75
0·014		9·50	7·31	4·96
0·016	10·10	7·33	5·88	3·75
0·018		5·96	4·36	2·36
0·020	6·05	4·10	3·05	
0·022		3·63	2·36	
0·024	3·83	2·83	1·90	
0·026		2·40		
0·028	2·56	1·86		
0·032	1·97			
0·036	1·30			
0·040	0·52			

TABLE IV

Hæmolyte—Piperidine

Concentration of piperidine in moles/litre	Time of hæmolysis in minutes, corpuscle concentration per litre			
	10	5·0	2·0	1·0
0·002				39·75
0·004	96·86	59·41	26·21	10·88
0·006		31·53	14·75	9·25
0·008	24·03	20·56	10·20	5·46
0·010	16·20	11·70	5·65	4·00
0·012	11·85	9·10	5·40	2·90
0·014	7·10	4·25	2·88	1·90
0·016	3·88	3·58	2·40	1·30
0·018	3·63			
0·020	2·41	2·16	1·25	.
0·024	1·78	1·65		

TABLE V

Hæmolyte—Ammonia

Concentration of ammonia moles/litre	Time of hæmolysis in minutes, corpuscle concentration per litre		
	10	5·0	2·0
0·2	27·25	19·60	13·93
0·26	21·46	15·10	10·88
0·30	18·46	12·20	9·36
0·36	14·80	10·10	7·63
0·40	12·15	8·86	6·15
0·44	10·40	8·00	
0·50	8·10	6·60	

TABLE VI

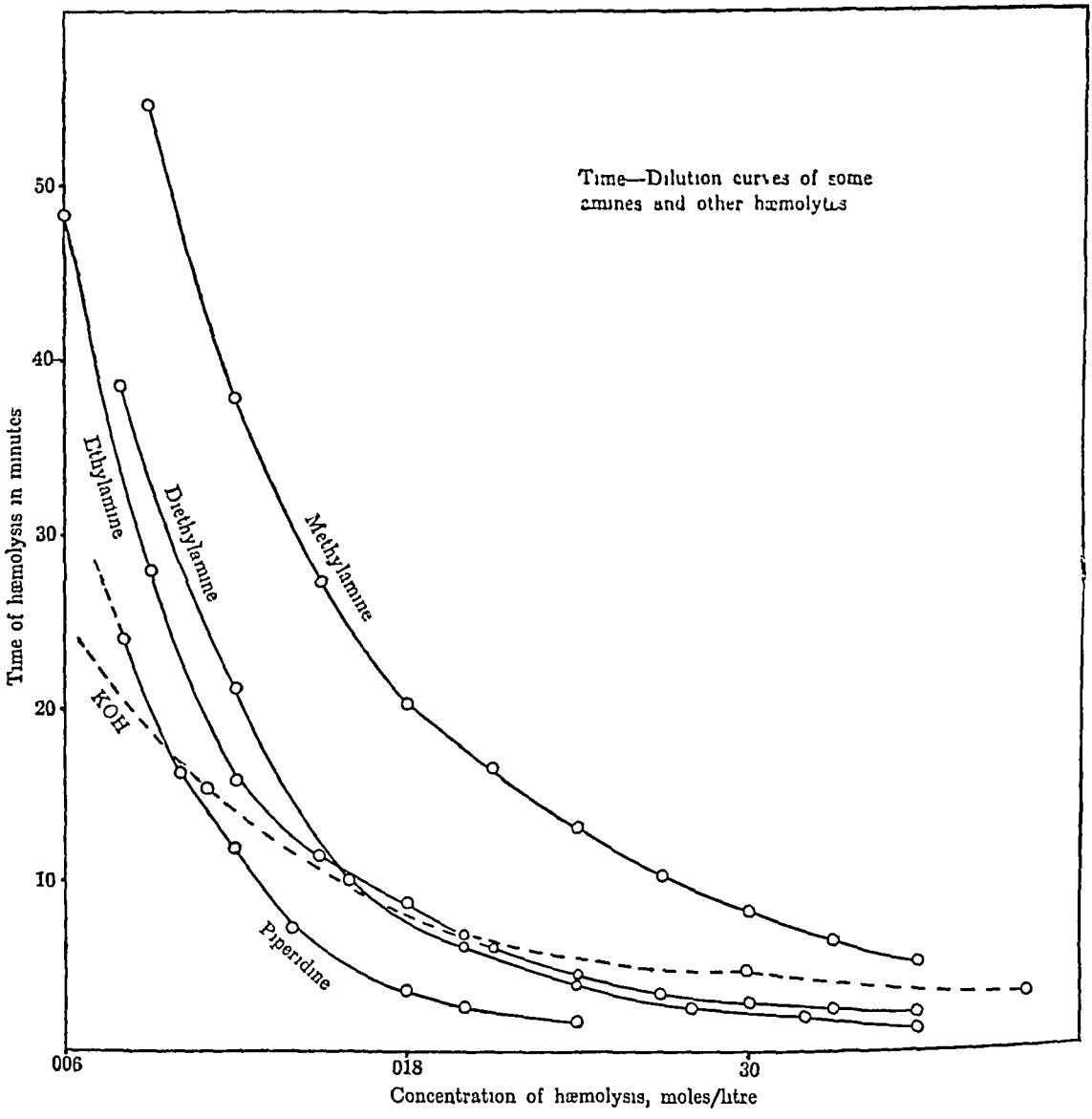
Hæmolyte—Potassium hydroxide

Concentration of caustic potash moles/litre	Time of hæmolysis in minutes, corpuscle concentration per litre		
	10	5·0	2·0
0·01	15·26	11·96	9·06
0·02	6·73	6·48	4·58
0·03	4·75	4·23	2·53
0·04	3·21	2·81	1·80
0·05	3·15	1·88	1·26

The results given in all the tables can be represented approximately by an equation of the type $C^m T = K$, where C and T are the concentrations and

times of hæmolysis, m a variable factor and K is a constant for each series In Graph 1 the data with different hæmolytes and 10 gs per litre corpuscles are graphically shown It will be observed that a comparative statement can

GRAPH 1



be made only with difficulty of the effect of the different alkaline substances on sheep's red blood corpuscles Thus the hæmolytic efficiency of the different substances are in the order KOH piperidine > ethylamine > diethylamine > methylamine, if the comparison is made at low concentrations of the hæmolytes where the time of hæmolysis is proportionately greater With higher concentrations of the hæmolytes some reversal occurs, notably that in the case of KOH The results in any case cannot be interpreted solely on the basis of the

concentration of OH' ions, though this view is found in the literature Thus Hober (1924) gives the following interesting table summarizing the known hæmolytic efficiency of several hæmolytic agents including some amines in relation to their conductivity, which gives a measure of their dissociation

TABLE VII

Hæmolyte	Mole per 100 litre (bases), mole per 100 litre (acids)	Molecular hæmolytic power	μ_{32}
Caustic potash	0·0075	133·0	210·0
Tetramethylammonium hydroxide	0·0060	166·0	208·0
Piperidine	0·0082	122 0	41 0
Dimethylamine	0 0130	76 9	31 0
Methylamine	0·0160	62·5	27 0
Ethylamine	0 0170	58·8	27 0
Propylamine	0·0170	58·8	23·9
Trimethylamine	0·0850	11 8	10 2
Piperazine	0·1980	5 0	9·1
Ammonia	0·2350	4 3	6 3
Hydrochloric acid	0·0500	20 00	120·0
Formic acid	0 1400	6·89	6 6
Acetic acid	0·3700	2·70	2·1
Propionic acid	0·6400	1 56	1 8
Butyric acid	0·8100	1 23	1 8
Valeric acid	0·8100	1 23	1 9
Caproic acid	0 6600	1 51	1 8

A perusal of this table will show that though there are well-marked exceptions, notable in the case of caustic potash and tetramethylammonium hydroxide, still a higher equivalent conductivity (indicated by μ_{32}) and a higher molecular hæmolytic power are closely associated This is especially true in the case of some of the fatty acids Curiously enough, the hæmolytic efficiency of the fatty acids does not run parallel to the effects which these substances produce on the surface tension at an air-water interface It will be remembered that Traube's theory of narcosis (Traube, 1908) rests on the assumption that permeability of a tissue or cell to narcotics is dependent on the ability of the

narcotics to depress the interfacial tension between the medium and the cell, but these results show that hæmolysis is dependent on some condition other than the effect on interfacial tension, or this effect is being overcome by some other effect of these substances As a matter of fact, in these particular cases the concentration of H° ions determines the hæmolytic efficiency In ordinary adsorption studies this type of results is often observed Thus we have recently obtained very similar results in a study of adsorption of these fatty acids by zirconium hydroxide gel It was observed that the stronger the acid was, the more it was adsorbed by zirconium hydroxide Now zirconium hydroxide is a basic substance and therefore with acids as the adsorbable solutes, the adsorption is markedly polar in nature Consequently the view has been put forward (Chakravarty and Sen, 1930) that in strongly polar adsorption, the effect of the capillary activity of the adsorbable solutes may be overcome, the chemical affinity existing between the adsorbent and the solute becoming predominant From this point of view it is believed that with fatty acids the adsorption by the cell may be of a chemical nature, the protein portion being more effective with a tendency to salt formation than the lipoids which show very little similar tendency With the amines, however, such a simple behaviour cannot be expected, because we are dealing with two adsorbents at the same time, namely the proteins as well as the lipoids, the latter being markedly peptized by the hydroxyl ions, and the differences in the effects of the different amines point to the fact that specific effects are coming into play Mention should, however, be made to a recent paper by Bodansky (1928) in which the order of the effectiveness of the fatty acids in hæmolysis is in accordance with surface tension theory An abstract of this paper only has been seen by the writers, and hence no discussion is given

Two points should be noticed in Table VII The so-called molecular hæmolytic power means really nothing unless the particular conditions of the experiment are stated Thus the efficiency of an hæmolyte depends on several factors, namely concentration of the corpuscles, the time allowed for the hæmolysis to be completed, the age of the corpuscles, the temperature of the solution, and probably on the total volume of the solution in which the experiment is carried out Christophers (1929*b*) in a recent paper considers that the concentration of the corpuscles and the concentration of an hæmolytic agent (acid or base) are directly proportional, and he states that molecular hæmolytic power is a fundamental constant The experiments on which he bases his observation were made with high concentrations of the corpuscles and the time allowed was also pretty high, namely three hours Apparently with these weaker concentrations of the hæmolyte, practically the whole of it was adsorbed and the results obtained were thus quite similar to those obtained by Oscar Gross, reference to whose work has already been made in an earlier page With lower concentrations of corpuscles, however, some deviations were observed. He would, we believe, have obtained more deviations if he

had decreased the time of hæmolysis even with higher concentrations of the cells Thus we have found the following results in this investigation —

TABLE VIII

Hæmolyte—Methylamine

Concentration of cells per litre mixture	Concentration of hæmolyte at different times of hæmolysis		
	15 minutes	*20 minutes*	*30 minutes*
10·0	0·022	0·018	0·014
5·0	0·016	0·013	0·0093
2·0	0·0125	0·0095	
1·0	0·0115	0·0087	

TABLE IX

Hæmolyte—Ethylamine

Corpuscle concentration	Concentration of hæmolyte at times		
	15 minutes	20 minutes	30 minutes
10·0	0·0126	0·0106	0·0086
5·0	0·0112	0·0088	
2·0	0·0102	0·0082	0·0058
1·0	0·0084	0·0064	0·0042

TABLE X

Hæmolyte—Diethylamine

Corpuscle concentration	Concentration of hæmolyte at times	
	10 minutes	15 minutes
10·0	0·016	0·0142
5·0	0·0137	0·0105
2·0	0·0115	0·009
1·0	0·0090	

TABLE XI

Hæmolyte—Piperidine

Corpuscle concentration	Concentration of hæmolyte at times		
	15 minutes	20 minutes	25 minutes
10·0	0·0105	0·009	0·0078
5·0	0·009	0·008	0·0068
2·0	0·006	0·005	0·004
1·0	0·0035	0·0025	0·002

TABLE XII

Hæmolyte—Ammonia

Corpuscle concentration	Concentration of hæmolyte at times	
	10 minutes	15 minutes
10·0	0·45	0·355
5·0	0·365	0·26
2·0	0·28	0·185

TABLE XIII

Hæmolyte—Caustic potash

Corpuscle concentration	Concentration of hæmolyte at times	
	5 minutes	10 minutes
10·0	0·029	0·015
5·0	0·025	0·0125
2·0	0·018	0·008

These results have been obtained by graphical interpolation and point to the fact that there is no direct proportionality between the concentrations of the cells and the hæmolyte when the time of hæmolysis is short Consequently we can have no such thing as molecular hæmolytic power unless we specify certain other conditions For these reasons we believe that the statement of Christopheis that the molecular hæmolytic power is an important and fundamental constant can not be justified

Effect of normal serum

In the previous paper it was shown that normal serum, if added together with the hæmolyte, has an inhibiting action on hæmolysis Some experiments have been made on the effect of normal serum on the hæmolytic behaviour of the amines, specially to find out if there is any change in the nature of the time-dilution curves, and in the following tables these results are shown in Table XIV, the final concentration of the corpuscles was 1 0 g per litre, and serum in a dilution of 1 in 10 was used —

Table XIV

Concentration of methylamine moles per litre	Time of hæmolysis with serum c c		
	0·05	0·10	0·15
0·009	29·1	36·36	42·26
0 012	21 33	24 75	30 55
0·015	14 5	17 9	20·85
0 018	11 2	13·76	16·91
0·021	9·3	10·7	12·1

Table XV contains results with 2 0 gs per litre corpuscles in presence of serum

Table XV

Concentration of methylamine moles/litre	Time of hæmolysis in minutes in presence of serum		
	0·05	0·10	0·15
0·009	29·63	35 95	41 55
0·012	21 6	25 0	28·0
0·015	16·6	18·33	21 41
0 018	11 95	13·78	15 93
0·021	10·06	11 13	12·7

Table XVI contains some results with ethylamine and serum The concentration of corpuscles is 1 0 g per litre

TABLE XVI

Concentration of ethylamine moles per litre	Time of hæmolysis in presence of serum c c		
	0·05	0·10	0·15
0·012	12·11		
0·018	6·28	7 28	
0 024	3 36	3 95	4·6
0·030	1 86	2·53	2·78
0 036	1·33	1 56	1 76
0·042	.	1 0	1 23
0·048	..	..	0·91

Table XVII contains data with 2 0 gs per litre corpuscle Other conditions are the same

TABLE XVII

Concentration of ethylamine moles/litre	Time of hæmolysis in presence of serum c c		
	0·05	0·10	0 15
0 012	15 8	18·13	22·46
0·018	7 5	8 86	10·4
0·024	4 03	4 71	6·01
0·030	2·3	2 66	3 06
0·036	1 41	1 7	1·88

Table XVIII contains some data with diethylamine and serum The concentration of corpuscles is one gramme per litre

In Table XIX, the effect of serum on diethylamine hæmolysis with two gs per litre corpuscles is shown

TABLE XVIII

Concentration of diethylamine moles/litre	Time of hæmolysis in presence of serum c c		
	0·05	0·10	0·15
0·008	16 33	19·51	23·43
0·010	10·48	13·43	15·86
0·012	7 35	8·88	10·86
0·016	3·9	4 85	6 03
0·020	2·41	2·7	3·01

TABLE XIX

Concentration of diethylamine moles per litre	Time of hæmolysis in presence of serum c c		
	0·05	0·10	0·15
0 008	26·18	30·6	33·93
0·012	13 85	15 93	16 83
0·016	6·73	8 18	8·98
0·020	3·88	4 3	5 2
0·024	2·05	2 6	2·65

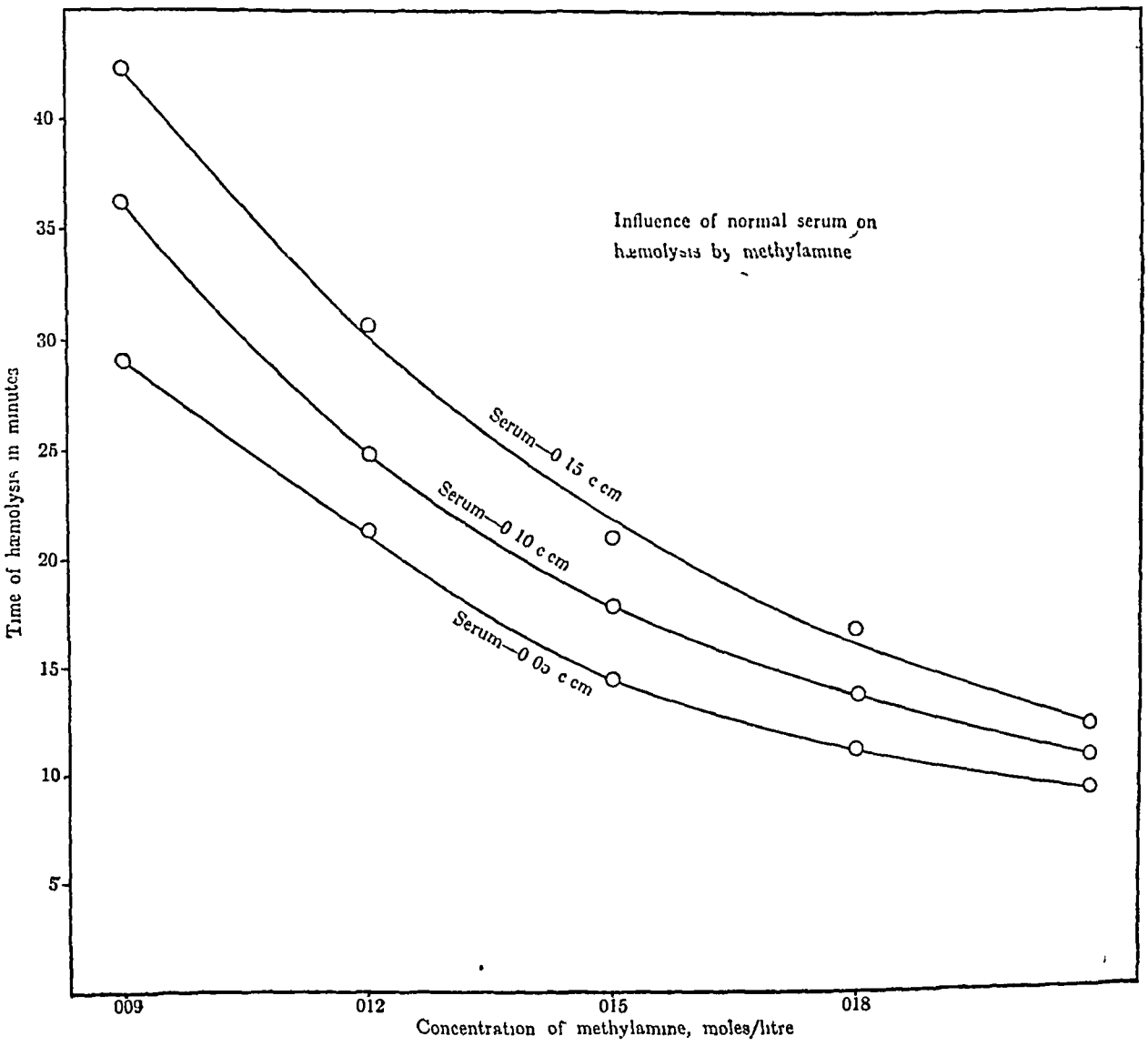
From all these tables it will be observed that normal serum, if added together with the hæmolyte, has a marked retarding effect on the hæmolytic efficiency of the amines With the same concentration of amines, the addition of increasing amounts of serum retards the process more and more, as can be easily seen from Tables XIV to XIX, which shows a behaviour very similar to that of alkali The nature of the time-dilution curves is not, however, changed, the curves being all of a parabolic nature Some of them are plotted in Graphs 2, 3 and 4

EFFECT OF AMINES ON OLEATE AND TAUROCHOLATE HÆMOLYSIS

It has already been noted in our previous paper that alkali gives a peculiar time-dilution curve when used with a chemical hæmolyte such as oleate, taurocholate, etc, and that under the conditions of the experiment, the presence of small amount of alkali accelerated the hæmolysis by *oleate, etc*, when the

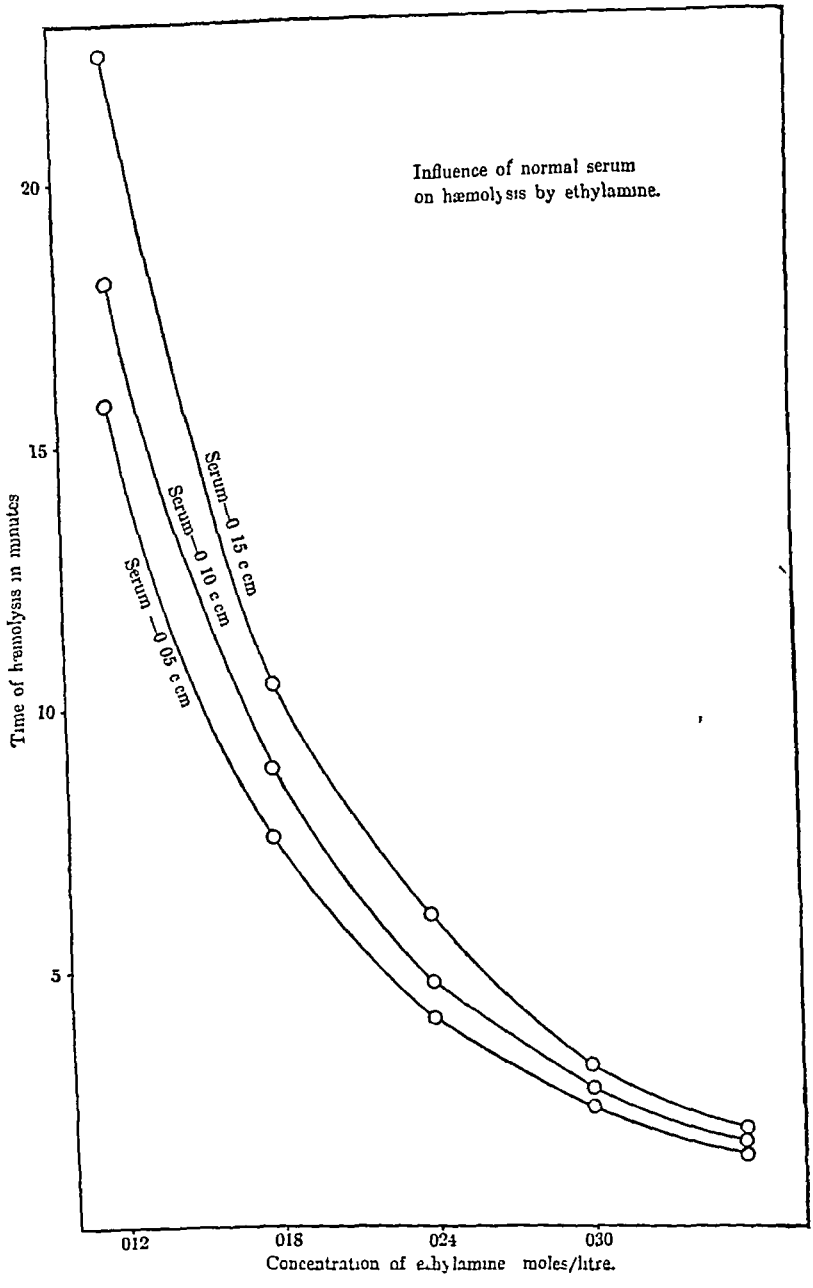
latter was present in small amounts but retarded the hæmolysis when the latter was present in higher amounts as compared to pure oleate hæmolysis It was also found that small amount of alkali accelerates the hæmolysis to a

GRAPH 2



great extent when it is added to sensitized corpuscles, depending on the concentration of the corpuscles and the time after which the alkali is added and it has been suggested by us that the peculiar behaviour of normal serum is due, at least partly, to its alkali content In order to verify this, we have sensitized the corpuscles with different chemical hæmolytes and then added very small amounts of these amines to see if there is any acceleration like that of alkali and serum Some of these amines being very weak alkalis will have very small concentrations of OH′ ions, and any acceleration by them of hæmolysis will go to verify our suggestion

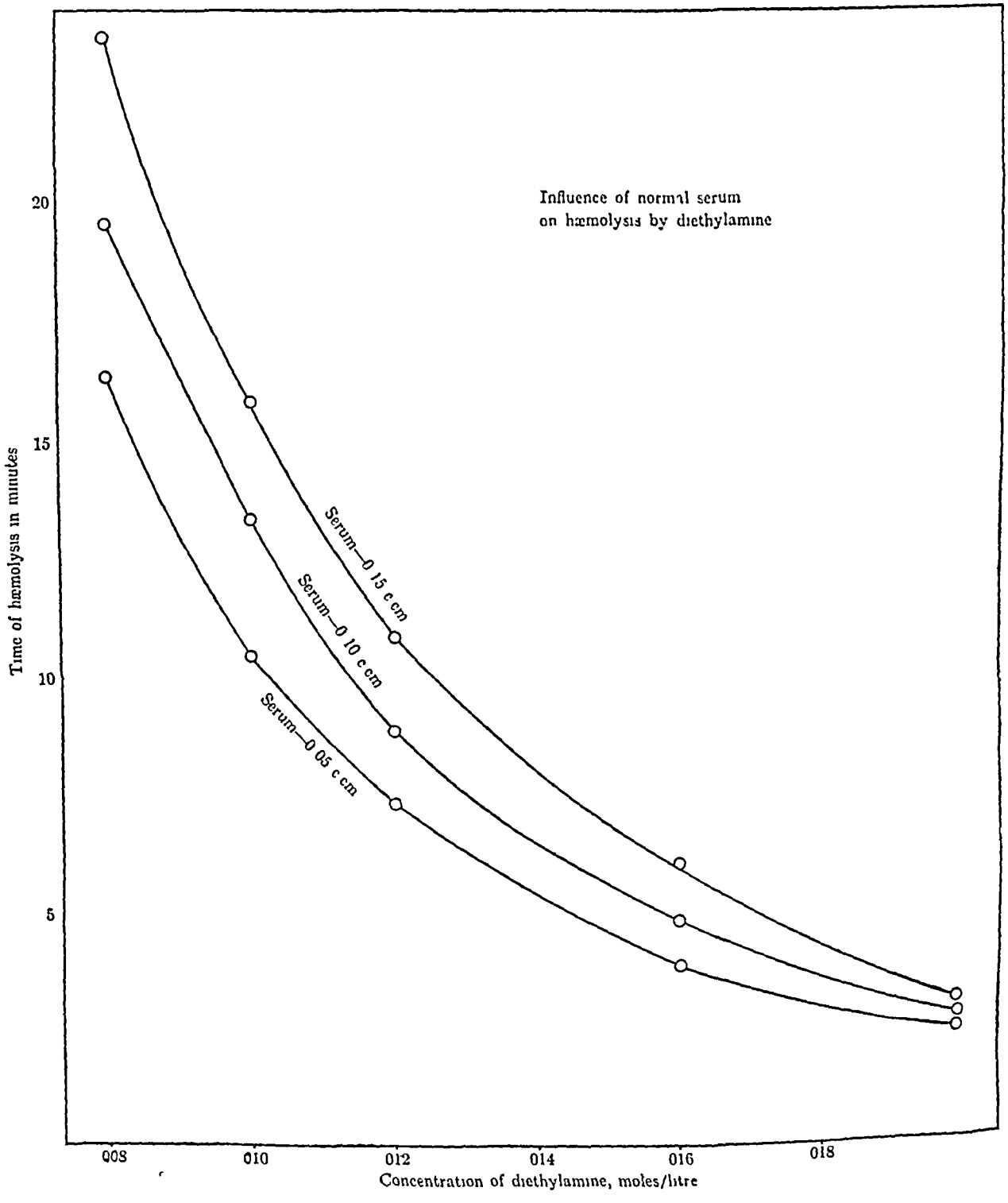
GRAPH 3



The following tables represent some results of oleate hæmolysis with ammonia, ethylamine and piperidine, the latter substances being added together with the oleate to the corpuscles The concentration of corpuscles is 1 0 g per litre of the mixture, the oleate concentration being 1 in 10,000

Table XXI represents the results with 0 001 moles of the ammonia and amines Other conditions remain the same

GRAPH 4



It should be stated that in some of these cases about 95 per cent hæmolysis took place very quickly, but owing to the 'ghost' formation, considerable time elapsed before the solution was quite clear

Table XXII shows results of taurocholate hæmolysis with these substances The original concentration of sodium taurocholate is 1 in 1,000 and of corpuscles 1 g per litre final mixture The total volume is 5 c c

TABLE XX

Amount of oleate in c c	Time of hæmolysis of the blank experiment	Time of hæmolysis with 0·002 mole		
		Ammonia	Ethylamine	Piperidine
0·2	47·2	102 33	46 3	28·93
0·4	14 3	118·05	40·5	24 4
0·6	3·75	117·1	42·7	22·73
0·8	2·1	100·9	24 1	20·46
1·0	1 13	86·6	24·5	17 25

TABLE XXI

Amount of oleate in c c	Time of hæmolysis of the blank experiment	Time of hæmolysis with 0·004 mole		
		Ammonia	Ethylamine	Piperidine
0·2	47 2	69·7	22 45	10·46
0·4	14·3	68·6	17 2	8·33
0·6	3·75	36·5	15 5	5·6
0·8	2·10	90% H in 60′	12·3	5 06
1·0	1 13	85% H in 50′	8·96	4 0

TABLE XXII

Amount of taurocholate c c	Time of hæmolysis of the blank experiment	Time of hæmolysis with 0·002 mole		
		Ammonia	Ethylamine	Piperidine
0·2	10% H in 3 hours	63·3	40·1	22·6
0·5	194 0	54 1	31 4	14 0
1 0	55·6	55 36	30 66	12 9
1 5	38·9	51 6	27 1	12·46
2·0	4 3	43·8	22·45	11·9

It will be observed from these tables that though the effect of these alkaline substances on oleate and taurocholate hæmolysis is mainly inhibitory in the concentrations studied, still considerable discrepancies are observed when each series is considered separately It will be remembered that in our previous studies with alkali and oleate, similar curious results were observed The present experiments are not, however, exhaustive enough to show definitely if we are dealing with some experimental error or some anomaly

It will be remembered that in the case of caustic soda hæmolysis and hæmolysis by serum, an acceleration was obtained by adding the serum or the small amount of alkali after the addition of the hæmolyte to the corpuscles The following table represents a set of results obtained by using a very dilute solution of piperidine with taurocholate as the hæmolyte The concentration of the corpuscles in the final mixture is 2 0 gs per litre and of taurocholate, original concentration 0 3 per cent The different amounts of piperidine were added half a minute after the addition of taurocholate to the corpuscles

TABLE XXIII

Time of hæmolysis with 0 3 *c c of taurocholate is* 17 6 *mins* 0 3 *c c of taurocholate have been used each time*

Piperidine moles per litre final mixture	Time of hæmolysis when the piperidine was added ½ minute after the addition of taurocholate
0·0	17 6
0·00002	31 16
0·00004	65 2
0 00006	150 0
0 00010	72 0
0 00012	15 0
0·00014	8·5
0 00016	Immediate H

Table XXIV shows the effect of addition of ammonia to taurocholate-sensitized corpuscles

TABLE XXIV

Concentration of ammonia moles per litre	Time of hæmolysis when the ammonia was added ½ minute after the addition of 0 3 c c taurocholate
0·0	17 6
0·00004	56 0
0 00006	98 0
0·00008	123 0
0 00012	57 0
0 00016	3 0

There was no hæmolysis in 2 hours with 0 00012 and 0 00016 moles of ammonia alone Table XXV gives the results of using ethylamine instead of ammonia

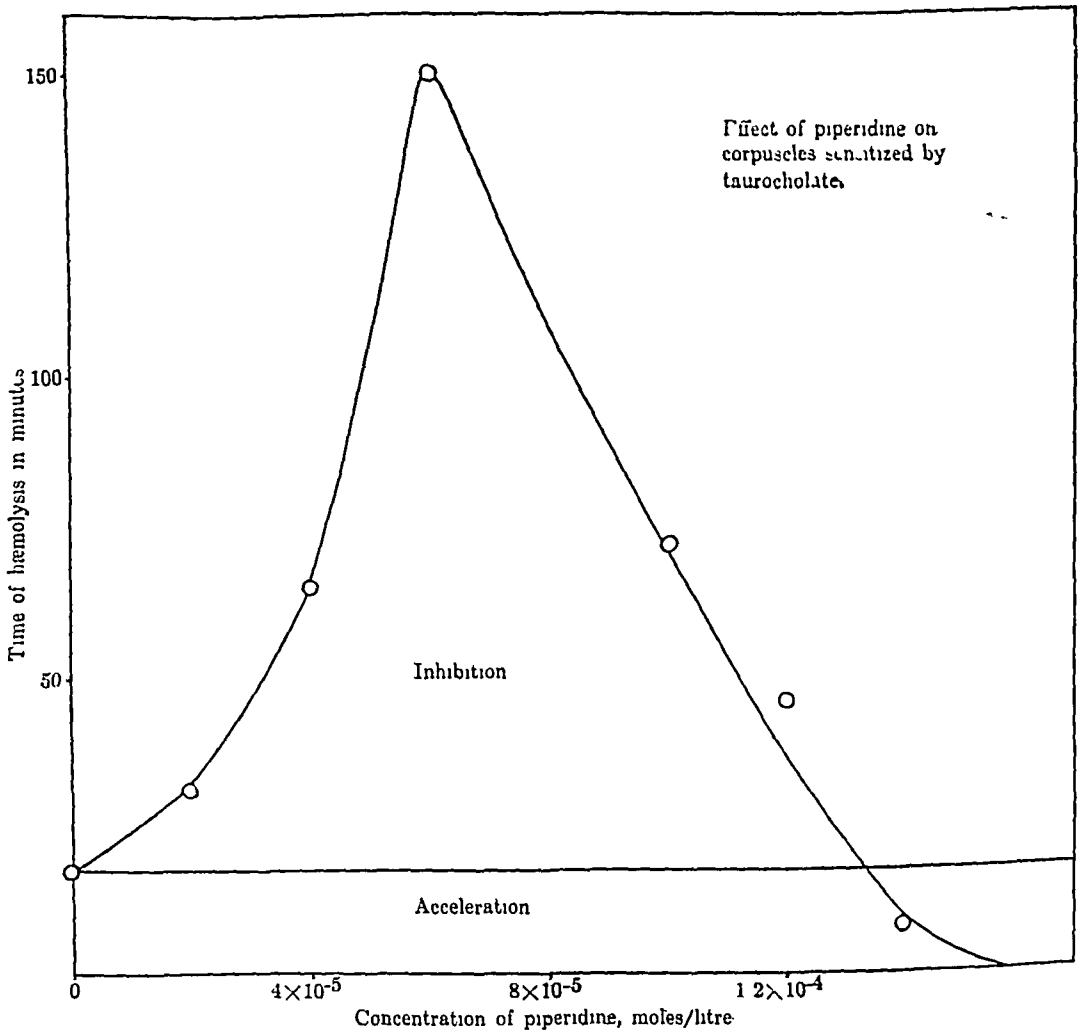
TABLE XXV

Concentration of ethylamine moles per litre	TIME OF HÆMOLYSIS WHEN *ETHYLAMINE* WAS ADDED	
	Together with taurocholate	½ minute after taurocholate
0·0	17 6	17 6
0·00002	23 0	24 6
0·00006	114 0	35 5
0·00008	104 0	20·45
0·00010	70 per cent H in 2 hours	4 0
0·00012	5 per cent H in 2 hours	Immediate

From a perusal of Tables XXIII to XXV it will be evident that there is a marked retardation as well as acceleration of hæmolysis depending on whether the ammonia, ethylamine, or piperidine is added before or after the addition of taurocholate The data with ammonia and piperidine are shown in Graphs 5 and 6 These results are therefore quite similar to those obtained previously by adding caustic alkalis or serum It is to be noticed that in the present cases, the amount of the alkaline substances added is very small Thus taking a particular case, say of ammonia, Table XXIV, we can consider that under the particular experimental conditions, the acceleration was found to take place with 0 0004 moles per litre of ammonia Of course if the time interval is increased to say 1 or more minutes, then the concentration of ammonia required to produce this acceleration would be much less Taking however this particular case, we can calculate the amount of OH′ ions which have been added Thus the degree of ionization would be given by the equation $\frac{a^2}{1-a} = \frac{K}{C}$, where a is the ionized amount, K is the dissociation constant and C is the concentration Since the value of K for ammonia (Bredig's) is $2\,3 \times 10$ the value of a on calculation comes to 0 215 approximately Consequently the concentration of OH′ ions would be $4 \times 10^{-4} \times 0\,215 = 8\,6 \times 10^{-5}$ This gives a pH of about 9 5 approximately to the solution This simple

calculation will show that it is possible to bring about the acceleration phenomenon by changing the reaction of the medium only to a slight extent Consequently the view put forward before that acceleration by serum may be due to its alkali content may be to a great extent true, because pH of the serum may be anything near about 7 3 to 7 5, and some acceleration is certainly possible owing to this change of the reaction It must be mentioned also that though by adding the small quantity of ammonia the pH of the

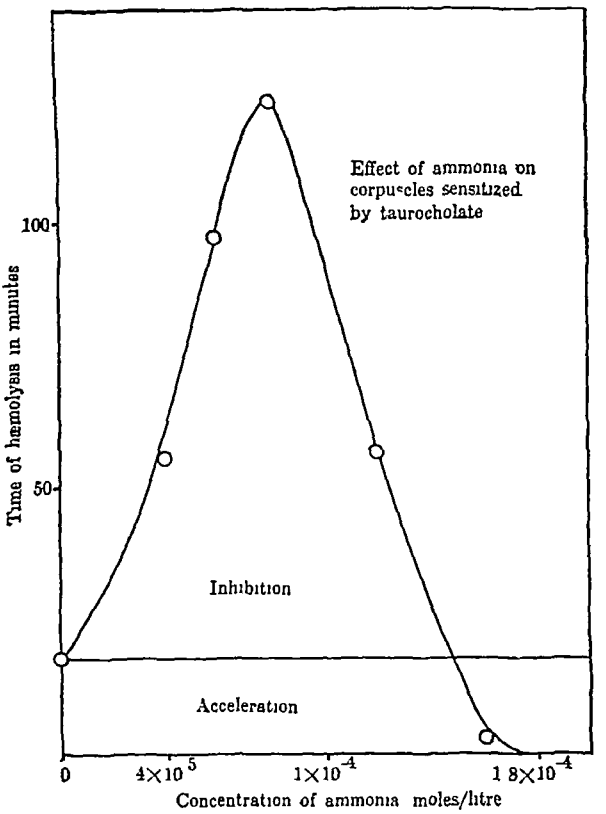
GRAPH 5



solution is brought up to 9 5, this is only the calculated value, as the resultant pH of the mixture will certainly come down to somewhere near 7 3 owing to the practically complete adsorption of ammonia by the corpuscles in such a dilute solution Hence from the experimental point of view, a slight change in the pH of the medium is sufficient to cause this acceleration of the hæmolysis of the sensitized corpuscles These results therefore support our view that the

accelerating action of normal serum on the hæmolysis of corpuscles by oleate or taurocholate is due to a change in the pH of the medium

GRAPH 6



SUMMARY

(1) An experimental study has been made of the hæmolytic effects of methylamine, ethylamine, diethylamine, piperidine, ammonia, and caustic potash on sheep's red blood corpuscles

(2) It is found that the concentration of the OH' ions in the solutions is not the only factor which determines the hæmolytic efficiency of these substances It is probable that specific effects are also brought into play

(3) Normal serum has a great inhibiting effect on the hæmolysis by amines if it is added before the addition of the hæmolytes This inhibitory effect increases with the increase in the quantity of the serum added, but the nature of the time-dilution curves remains similar

(4) The addition of the amines together with oleate or taurocholate inhibits the hæmolytic efficiency of the latter substances considerably, but if they are added to the corpuscles after the oleate and taurocholate have been

mixed with the corpuscles, then a great acceleration may be obtained under suitable conditions This phenomenon is quite analogous to the acceleration phenomenon with normal serum studied in previous papers

(5) Calculation shows that after the addition of very dilute alkali to corpuscles previously sensitized by oleate or taurocholate, a slight increase in pH to the alkaline side is sufficient to cause the acceleration of hæmolysis Consequently this supports the view, previously given by the writers, that the accelerating effect of normal serum may be due, at least in part, to the alkali content of the serum

In conclusion, we wish to express our best thanks to Mr F Waie, FRCVS, IVS, Director, Imperial Institute of Veterinary Research, for his kind interest in this paper

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FURTHER RESEARCHES ON STONE

Part VIII

BY

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WITHIN recent years a large amount of experimental evidence has accumulated tending to show that 'stone' is a deficiency disease This evidence has been derived from experimentation on rats animals said to be 'very prone to stone' Dr Swift-Jolly (1929) has, therefore, advised caution in applying to man the results of observations made on rats 'It would,' he says, 'be a distinct advance if it could be shown that animals normally free from the disease develop stone when kept on a deficient diet' The present paper provides evidence not only that *deficiently-fed rats* are 'very prone to stone,' but that *well-fed rats* are 'normally free from the disease'

Freedom of well-fed rats from stone

During the past three years complete post-mortem examinations have been made in 844 well-fed albino rats Of these 416 were fed on complete, synthetic diets, urinary calculus was not present in any of them This experience is similar to that of van Leersum (1927) who has sought in vain for stone in hundreds of rats fed on complete, synthetic diets The remaining 428 were fed on our stock diet

They were of all ages from 30 days onwards and of body-weights ranging from 10 to 300 grammes They were killed with the dual object of establishing

normal standards of organ-weights and of determining the incidence of calculous disease in our stock A careful search was made in each of these animals for urinary calculus it was invariably absent

As a result, therefore, of the post-mortem examination of 844 animals it can be stated with assurance that well-fed albino rats,* living under conditions of perfect sanitation, do not develop urinary calculus in these laboratories they are 'normally free from the disease' The stock diet consists of whole-wheat flour chapattis lightly smeared with butter, the hard crust of white loaves, sprouted *gram* (legume), fresh, raw carrots and cabbage *ad libitum*, diluted whole milk, an occasional small ration of raw meat, and fresh water in abundance On this diet urinary calculus does not arise in this climate and at this altitude (6,000 feet)

On the other hand, calculous disease occurs with great frequency in albino rats fed on deficient and ill-balanced diets In the course of our work on this subject we have encountered stone on 205 occasions amongst 884 deficiently-fed animals The contrast afforded by well-fed and deficiently-fed rats is thus as follows —

In 844 *well-fed rats* there was no case of stone

In 884 *deficiently-fed rats* there were 205 cases of stone

This difference is, statistically speaking, very significant, it leaves little room for doubt that in albino rats urinary calculus is a 'disease of faulty nutrition' This term is preferred to that of 'deficiency disease,' since a number of dietetic factors have been found to exercise a stone-producing influence in rats These factors are of two orders those of deficiency and those of excess of certain food-constituents The former are *negative factors*, the latter *positive factors* The *negative factors*, so far found, are deficiency of fat-soluble vitamins and deficiency of phosphates† The positive factors are excess of lime, and some unknown agent present in varying amounts in different cereal grains To the latter there must be added infection of the urinary tract The permutations and combinations of these factors are many, but so far as our work has gone it would seem to show that positive stone-producing factors are innocuous in the presence of a sufficiency of fat-soluble vitamins and phosphates The evidence on which these statements are based is to be found in our previous reports on this subject (McCarrison, 1927*a*, *b*, *c*, 1928, and 1930*a*, *b*, *c*) Such causes of stone as foreign bodies and stagnation of urine in the urinary tract are outside the scope of our investigations which relate only to the influence of dietary conditions on stone-production

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* Our stock rats live in straw-filled cages The straw is frequently changed The animals are fond of nibbling the straw which is to be regarded as part of their diet

† The work dealing with deficiency of phosphates as a stone-producing agency will appear in a subsequent issue of this Journal

Effect of the tropical, solar rays on the incidence of stone

It is now known that *an excess of vitamin D* in the food is amongst the positive stone-producing agencies referred to above, and it has been suggested (Dixon and Hoyle, 1928) that the high incidence of urinary calculus in deficiently-fed natives of the tropics may be related to the manufacture of 'large amounts of active ergosterol in their widely exposed epidermis, since the solar rays have high actinic value' I have, however, been unable to provide experimental evidence in support of this view Two groups of 18 young albino rats—9 males and 9 females in each—were fed on a diet consisting of whole-wheat flour 90 parts, linseed oil 8 parts, calcium phosphate 1 part, sodium chloride 1 part, and water This diet had previously been found to cause a fair incidence of phosphatic calculus (McCarrison, 1928) Each animal was confined in a separate cage One group was kept wholly in the dark, the animals being fed and their cages cleaned at dusk The other group was exposed daily to the direct rays of the mid-day and mid-summer sun, which at this altitude are of high actinic value The exposures were gradually increased from half-an-hour to 2 hours In order further to increase the production of vitamin D the backs and flanks of the animals, in places accessible to licking, were smeared with gingelly (sesame) oil The experiment was continued for 8 months when the surviving animals were killed Amongst the rats exposed to sunlight there were two cases of vesical calculus (the stones weighing 32 and 126 grammes respectively and being of the ammonium-magnesium-phosphate variety), one case of cystitis-without-stone also occurred in this group Amongst those kept in the dark there were also two cases of vesical calculus of the ammonium-magnesium-phosphate variety (the stones weighing 20 and 38 2 mg respectively), one of these was associated with severe pyonephrosis and cystitis, the other had no apparent cystitis nor other obvious pathological state of the urinary tract The incidence of calculous disease in the two groups was, therefore, the same, and unless the greater aggregate weight of the calculi in the 'sunlight group' is to be regarded as significant there was no difference between them In view of the effect of sunlight in protecting albino rats against rickets (Hess *et al*, 1922) there would seem to be no doubt that vitamin D is thereby produced The amount so produced in the above experiment did not, however, increase the incidence of stone

The experiments

In a previous paper (McCarrison, 1930*a*) attention was drawn to the effects of slaked lime—when it formed part of a diet deficient in fat-soluble vitamins and phosphates—in favouring the development of urinary calculus in rats It was found that while the incidence of the disease was 16 6 per cent in rats fed on a diet of white bread, dried yeast and distilled water, it was 42 8 per cent when slaked lime, in the proportion of approximately 3 grains per rat per

day, was added to this diet, the experiments having been carried out during the summer months With the object of developing this observation further a series of experiments was carried out, during the past year, the results of which have now to be recorded

Seven groups of 24 young rats were employed, the sexes being as far as possible equally distributed in each group Details regarding their sex and body-weight are given in the accompanying Tables (I to VII) Each animal was confined in a separate, screened cage (1 foot × 1 foot × 1 foot) under conditions of scrupulous cleanliness

The diets used were as follows —

A A basal diet consisting of white bread 97 parts, dried yeast 3 parts and distilled water Two groups of rats were fed on this diet one in the spring and summer of 1929 (Table I) the other in the autumn and winter of 1929-30 (Table II)

B The basal diet *plus* 3 grains (approximately) of slaked lime per rat per day Two groups of rats were fed on this diet one during the spring and summer of 1929 (Table III), the other during the autumn and winter of 1929-30 (Table IV)

C The basal diet *plus* 3, decreased to 1 5, grains of slaked lime per rat per day One group of rats was fed on this diet during the autumn, winter and spring of 1929-30 (Table V)

D As in C but with two parts of gingelly (sesame) oil substituted for 2 parts of the white bread One group of rats was fed on this diet during the late autumn, winter, spring and early summer of 1929-30 (Table VI)

E As in D but with radiostoleum added to the gingelly oil in the proportion of 1 drop of radiostoleum to every 5 c c of the oil One group of rats was fed on this diet during the late autumn, winter, spring and early summer of 1929-30 (Table VII)

A trace of iodine was added to some of these diets, its presence or absence had no effect on the incidence of stone The basal diet—A—has the following as its chief defects deficiency of vitamins A, C and D, and deficiency of phosphates The addition of lime to this diet introduces another factor an excess of lime, involving great imbalance between lime and phosphates The further addition of the vitamin-poor vegetable oil tends to increase the relative deficiency of fat-soluble vitamins, while the addition of radiostoleum—which contains both vitamin A and vitamin D—is intended to rectify the deficiency of these factors, leaving the calcium-phosphate imbalance as the chief fault of DIET E The deficiency of vitamin C in all these diets is, for the present,

neglected, since rats seem to do well enough without it though they do better with it

The lime was added to the diet in the proportion of 5 grains to every 20 grammes of the food-mixture When present in this concentration the daily average food-consumption was such that approximately 3 grains of lime were ingested by each rat daily But the food-consumption by different animals differed, while that of individuals varied from day to day, 3 grains is, therefore, an approximate estimate of the actual amount of lime ingested by each rat daily The animals fed on DIET B consumed this amount of lime throughout the whole course of the experiments Those fed on DIETS C, D and E consumed this amount during the first 75 days of the experiments, *thereafter the concentration* of lime was reduced to 2 grains per 20 grammes of the diet This reduction was followed by an improvement in appetite, so that from the seventy-sixth day onward each rat ingested approximately 1 5 grains of lime daily

The amount of radiostoleum ingested by the rats fed on DIET E was approximately 0 06 of a drop daily

There are thus for comparison (*a*) two groups of rats fed, during *the spring and summer months,* on the basal diet with and without lime (Tables I and III), (*b*) two groups of rats fed, during *the autumn and winter months,* on the basal diet with and without lime (Tables II and IV), (*c*) two groups of rats fed, during *the autumn, winter and early spring months,* on the basal diet *plus* lime with and without gingelly oil (Tables V and VI), and, (*d*) two groups of rats fed, during *the autumn, winter and spring,* on the basal diet *plus* lime and gingelly oil with and without radiostoleum (Tables VI and VII) The effects of season, of lime in larger and smaller dosage, of vitamin-poor vegetable oil, and of radiostoleum were thus studied

The results of these experiments are set out in Tables I to VII and are summarized in Table VIII

Results of the experiments.

The basal diet—A—of bread, yeast and water without added lime, admitted of fair growth in animals escaping infection (Chart 1) But of these there were relatively few The tendency to infection of the pulmonary and intestinal tracts was very great and the resultant mortality high, especially during the summer months (Tables I and II) The incidence of vesical calculus was 14 6 per cent and of ureteral calculus 2 per cent, renal calculus did not occur (Table VIII) Cystitis was present in 12 5 per cent of the animals, hydronephrosis in 2 per cent, and dilated ureters in 4 per cent Pyonephrosis was conspicuous by its absence In 5 of the 7 cases (Tables I and II) in which vesical calculus occurred, the calculi existed as phosphatic 'gravel', in the two remaining cases well-formed 'stones' were present The

2435	M	53	88	63	?	—	—	—	—	—	—	—	—	—
2436	F	54	84	96	G U Disease	++	—	—	—	—	—	—	G +	—
2437	F	57	65	67	Asthenia	—	—	—	—	—	—	—	G +	—
2438	F	56	66	77	Enteritis, abscess	—	—	—	—	—	—	—	—	—
2439	M	52	48	26	Pneumonia	—	—	—	—	—	—	—	—	—
2440	M	52	97	137	Enteritis, ascitis	—	—	—	—	—	—	—	G ++	—
2441	F	51	50	135	Inanition	—	—	—	—	—	—	—	—	—
2442	F	50	52	37	Bi -pneumonia	—	—	—	—	—	—	—	—	—
2443	F	56	58	60	Pneumonia	—	—	—	—	—	—	—	—	—
2444	M	50	79	106	G I Disease	—	—	—	—	—	—	—	—	—
2145	M	60	57	33	Inanition	—	—	—	—	—	—	—	—	—
2446	M	53	90	94	G U Disease, abscess neck	—	—	—	—	—	—	—	+	35 0
Totals		1,214	1,822	1,873		2	—	—	—	—	—		5	
Averages		50 6	75 9	78 0										

The stone occurring in rat No 2446 was of the ammonium-magnesium-phosphate variety Urine of gravel cases showed ammonium-magnesium-phosphate crystals No 2437 also showed oxalate crystals

Note—Symbols for all Tables (I to VII)

G U Disease = Disease of the urinary tract G I Disease = Disease of the gastro-intestinal tract

I = Incrusted cystitis

G + = 'Gravel' not formed stone + = Small in amount in case of calculus material, moderately severe in case of cystitis, etc

++ = Plentiful, severe +++ = Very plentiful, very severe —=Not present

Table II

Giving details regarding 24 rats fed on a diet consisting of white bread 97 parts, dried yeast 3 parts and distilled water

Experiment commenced 13-8-1929, ended 4-2-1930

Laboratory number of rat	Sex	Original body-weight gs	Final body-weight gs	Days under experiment	Cause of death	Cystitis	Pyo-nephrosis	Hydro-nephrosis	Dilated ureters	Stone in ureters	Stone in kidney	Weight of kidney stone mg	Stone-in-the-bladder	Weight of bladder stone mg
2621	M	41	88	73	Intestinal obstruction	—	—	—	—	—	—	—	—	—
2622	F	48	61	52	Hepatic disease	—	—	—	—	—	—	—	—	—
2623	M	43	140	175	Killed	—	—	—	—	—	—	—	—	—
2624	F	44	107	112	G I Dystrophy	—	—	—	—	—	—	—	—	—
2625	M	41	97	98	Pneumonia, pericarditis	—	—	—	—	—	—	—	—	—
2626	F	46	67	117	Pneumonia	—	—	—	—	—	—	—	—	—
2627	M	48	129	175	Killed	—	—	—	—	—	—	—	—	—
2628	F	48	88	134	G U Disease	+	—	—	—	—	—	—	G +	—
2629	M	48	115	89	Pneumonia	—	—	—	—	—	—	—	—	—
2630	F	47	76	132	Do	—	—	—	—	—	—	—	—	—
2631	M	45	68	101	Do	—	—	—	—	—	—	—	—	—

2632	I	44	90	145	Do	—	—	—	—	—	—	—	—	—
2597	M	50	146	175	Killed	—	—	—	—	—	—	—	—	—
2598	F	12	92	113	G U Disease	I +++	—	+	++	G +	—	—	++	57 0
2599	M	47	156	175	Killed	—	—	—	—	—	—	—	—	—
2600	F	43	95	150	G U Disease	++	—	—	+	—	—	—	—	—
2601	M	49	150	175	Killed	—	—	—	—	—	—	—	—	—
2602	I	40	119	175	Do	—	—	—	—	—	—	—	—	—
2603	M	48	157	175	Do	—	—	—	—	—	—	—	—	—
2604	F	50	130	175	Do	—	—	—	—	—	—	—	—	—
2605	M	45	161	175	Do	—	—	—	—	—	—	—	—	—
2606	F	43	90	106	G U Disease	++	—	—	—	—	—	—	—	—
2607	M	45	116	175	Killed	—	—	—	—	—	—	—	—	—
2608	F	12	128	175	Do	—	—	—	—	—	—	—	—	—
Totals		1 087	2,609	3,347		4	—	1	2	1	—	—	2	—
Averages		45 3	111 2	139·5										

The stone occurring in rat No 2598 was of the ammonium-magnesium-phosphate variety Urine of gravel case (No 2628) showed ammonium-magnesium-phosphate crystals

Table III

Giving details of 24 rats fed on a diet consisting of white bread 97 parts, dried yeast 3 parts, slaked lime 3 grams per rat per day, and distilled water

Experiment commenced 5-4-1929, ended 12-10-1929

Laboratory number of rat	Sex	Original body-weight gs	Final body-weight gs	Days under experiment	Cause of death	Cystitis	Pyonephrosis	Hydronephrosis	Dilated ureters	Stone in ureters	Stone in kidney	Weight of kidney stone mg	Stone-in-the-bladder	Weight of bladder stone mg
2531	M	45	72	86	Asthenia	—	—	—	—	—	—	—	—	—
2532	F	42	129	189	Killed	—	—	—	—	—	—	—	G +	—
2533	M	41	105	90	Pneumonia	—	—	—	—	—	—	—	—	—
2534	F	42	89	123	G U Disease, hydrothorax	—	+	+	+	—	—	—	++	—
2535	F	48	113	134	G U Disease	I +	—	—	—	—	—	—	—	—
2536	F	41	80	115	Do	++	—	++	++	—	—	—	G ++	—
2537	F	49	116	173	Do	I +	—	—	+	—	—	—	G ++	—
2538	M	42	54	95	G I Disease	—	—	—	—	—	—	—	—	—
2539	M	42	72	52	Do	—	—	—	—	—	—	—	—	—
2540	F	42	68	66	Asthenia?	—	—	—	—	—	—	—	—	—
2541	M	50	93	118	G U Disease	+	+	—	+	—	G +	—	G ++	—

2542	M	48	99	118	Do	+ +	—	—	++	—	—	—	G + +	--
2543	M	47	61	76	Do	I +	++	—	+	—	—	—	—	—
2544	F	46	85	128	G U Disease, enteritis	++	—	—	+	—	—	—	G +	—
2545	M	41	47	55	Enteritis	—	—	—	—	—	—	—	—	—
2546	F	44	88	172	G U Disease	+	++	—	+	+	+	—	++	38·6
2547	F	45	110	174	?	—	—	—	—	—	—	—	—	—
2548	F	41	62	117	G U *Disease*, bi -pneumonia	++	—	—	—	—	—	—	G ++	—
2549	F	50	56	105	Pneumonia	—	—	—	—	—	—	—	—	—
2550	M	42	43	19	Do	—	—	—	—	—	—	—	—	—
2551	M	11	142	191	Killed	—	—	—	—	—	—	—	—	—
2552	F	45	95	186	G U Disease	++	—	—	—	—	G +	—	G +	—
2553	F	47	111	191	Killed	—	—	—	—	—	—	—	—	—
2554	F	43	117	159	G U *Disease*	++	—	+	++	—	—	—	—	—
Totals		1,061	2,107	2,932		11	4	2	9	1	3	—	10	
Averages		41·3	87·8	122·2										

Calculous deposits in all cases were made up of calcium carbonate or hydroxide or both

TABLE IV

4 rats fed on a diet consisting of white bread 97 parts, dried yeast 3 parts, calcined lime 3 grains per rat per day, and distilled water

EXPERIMENT COMMENCED 21-8-1929, ENDED 4-2-1930

Laboratory number of rat	Sex	Original body-weight gs	Final body-weight gs.	Days under experiment	Cause of death	Cystitis	Pyo-nephrosis	Hydro-nephrosis	Dilated ureters	Stone in ureters	Stone in kidney	Weight of kidney stone mg	Stone-in-the-bladder	Weight of bladder stone mg
2633	M	43	58	64	G U Disease, ascitis	—	—	—	+	—	—	—	G ++	146 0
2634	F.	44	47	62	Inanition	—	—	—	—	—	—	—	—	—
2635	M	47	53	77	Pneumonia	—	—	—	—	—	—	—	—	—
2636	F	44	59	119	G U Disease	I +	+	—	++	—	—	—	++	90·0
2637	M	44	64	160	Do	++	—	—	—	G +	G +	—	G +	—
2638	F	49	63	110	?	—	—	—	—	—	—	—	—	—
2639	M	47	50	67	G U Disease	I +	+	—	+	—	—	—	—	—
2640	F	42	86	119	G U Disease, ascitis	++	—	+	++	—	—	—	+++	475 0
2641	M	45	44	145	G U. Disease	+	—	—	++	—	++++	1,530	+++	555 0
2642	F	47	35	11	Pneumonia	—	—	—	—	—	—	—	—	—
2643	M	41	60	87	Pneumonia, pleurisy, ascitis	++	+	—	+	—	—	—	++	'117 0

2644	F	50	71	55	Pernicious anæmia	—	—	—	—	—	—	—	—	—	
2609	M	49	97	67	G U Disease	—	—	—	—	—	—	—	G +	—	
2610	F	47	103	167	Killed	++	—	—	—	—	—	—	G +	—	
2611	M	49	94	167	Do	+	—	+	+	—	—	—	+++	638 0	
2612	F	46	80	167	Do	+	+++	—	+	—	—	-	++	212·0	
2613	M	42	93	113	G U Disease, ascitis	—	—	+	++	G +	—	—	++	266 0	
2614	F	48	87	94	G U Disease	—	—	—	—	—	—	—	G +	—	
2615	M	50	70	107	Do	++	++	—	++	G +	++	39	++++	1,250 0	
2616	F	41	78	75	G U Disease ascites	++	+	—	+	—	—	—	+	43 0	
2617	M	50	92	83	Do	+	—	—	++	—	+	24	++	139·0	
2618	F	41	104	167	Killed	—	—	—	—	—	—	—	+++	715 0	
2619	M	41	110	167	Do	++	—	—	—	—	—	—	—	—	
2620	F	40	56	115	G U Disease	I +	—	—	—	—	—	—	++	88 0	
Totals		1,087	1,754	2,565		14	6	3	12	3	4		17		
Averages		45 3	73 1	106 9											

Vesical stones formed of calcium carbonate occurred in Nos 2636, 2643, 2611, 2612, 2615, 2616, 2618 and 2620
Vesical stones formed of calcium hydroxide occurred in Nos 2633, 2641 and 2617 Mixed calcium carbonate and calcium hydroxide vesical stones occurred in Nos 2640 and 2613 All renal stones—Nos 2641, 2615 and 2617—were composed of calcium carbonate

TABLE VI

Giving details of 24 rats fed on a diet of white bread 95 parts, dried yeast 3 parts, gingelly oil 2 parts, slaked lime 3 to 1½ grams per rat per day, and distilled water

EXPERIMENT COMMENCED 25-10-1929, ENDED 30-6-1930

Laboratory number of rat	Sex	Original body-weight gs	Final body-weight gs	Days under experiment	Cause of death	Cystitis	Pyonephrosis	Hydronephrosis	Dilated ureters	Stone in ureters	Stone in kidney	Weight of kidney stone mg	Stone-in-the-bladder	Weight of bladder stone mg
2753	F	56	36	41	Inanition	+	—	—	—	—	—	—	++	119·0
2754	F	51	36	70	G U Disease	++	++	—	++	—	—	—	++	84·0
2755	F	64	58	71	Do	+	—	—	—	—	G +	—	G +	15·0
2756	F	64	135	247	Killed	—	—	—	—	—	—	—	—	—
2757	F	63	48	65	Pneumonia	—	+++	—	+++	—	—	—	—	—
2758	F	65	88	155	G U Disease	+++	+++	—	+	—	—	—	G +	—
2759	F	70	82	144	Do	+++	+++	—	+++	—	—	—	—	—
2760	F	68	56	72	G U Disease, ascitis	++	++	—	++	—	—	—	G +	—
2761	F	62	49	68	G U Disease	+	+++	—	++	—	—	—	++	28·0
2762	F	71	70	44	Pneumonia, ascitis	—	—	+	+	—	—	—	++	48·0
2763	F	63	—	—	Used for biochemical studies									

2764	F	58	51	76	Pneumonia	—	—	—	—	—	—	—	—	—
2765	F	55	41	71	G U Disease	+	—	—	++	+	++	10	++	80·0
2766	M	75	60	68	Do	+	—	—	—	—	++	44	++	139 0
2767	M	75	65	18	Inanition	—	—	—	—	—	—	—	G +	10 0
2768	M	58	40	40	G U Disease	+	—	—	+++	G +	—	—	++	36 0
2769	M	74	40	46	Do	++	+++	—	+	—	—	—	++	142 0
2770	M	69	48	77	G U Disease, abscess neck	+	+++	—	++	+	++	84	++	46·0
2771	M	69	37	42	Pneumonia	++	—	+	+	G +	G +	15	++	39·0
2772	M	71	—	—	Used for biochemical studies									
2773	M	67	13	51	G U Disease	+	++	—	+	G +	G +	10	++	52 0
2774	M	75	63	25	Do	+	—	+	++	+	—	—	++	20 0
2775	M	51	40	50	Do	++	++	—	++	—	G +	—	++	114·0
2776	M	53	25	55	Do	++	++	—	++	+	—	—	++	112 0
Totals		1,548	1,436	1,599		17	11	3	16	7	7		18	
Averages		61 5	55 0	72·7										

No 2771 had a large collection of calcium carbonate, weighing 114 mg, in the prepuce and occluding the urinary outflow
Calcium carbonate stones occurred in the bladder in rats Nos 2753, 2761, 2768, 2770 and 2774
Calcium hydroxide stones occurred in the bladder in rats Nos 2762 and 2771
Mixed calcium carbonate and calcium hydroxide stones occurred in the bladder in rats Nos 2755, 2765, 2766, 2767, 2769, 2773, 2775 and 2776
One ureteral stone (rat No 2765) was analysed, it was calcium carbonate
Two kidney stones (Nos 2765 and 2773) were calcium carbonate, two (Nos 2766 and 2771) were calcium hydroxide and the other (No 2770) was mixed

Table VII

Giving details of 24 rats fed on a diet of white bread 95 parts, dried yeast 3 parts, radiostoleum in gingelly oil 2 parts, slaked lime 3 to 1½ grains per rat per day, and distilled water

Experiment commenced 25-10-1929, ended 30-6-1930

Laboratory number of rat	Sex	Original body-weight gs	Final body-weight gs	Days under experiment	Cause of death	Cystitis	Pyo-nephrosis	Hydro-nephrosis	Dilated ureters	Stone in ureters	Stone in kidney	Weight of kidney stone mg	Stone-in-the-bladder	Weight of bladder stone mg
2705	F	55	130	248	Killed	—	—	—	—	—	—	—	—	—
2706	F	58	150	248	Do	—	—	—	—	—	—	—	—	—
2707	F	72	140	248	Do	—	—	—	—	—	—	—	—	—
2708	F	63	56	86	G U Disease	++	—	—	++	—	—	—	++	105 0
2709	F	67	137	248	Killed	—	—	—	—	—	—	—	—	—
2710	F	65	129	248	Do	—	—	—	—	—	—	—	—	—
2711	F	68	139	248	Do	—	—	—	—	—	—	—	—	—
2712	F	63	40	70	Pneumonia	—	—	—	—	—	—	—	—	—
2713	F	64	55	82	Do	—	—	—	—	—	—	—	—	—
2714	F	Escaped from cage, excluded												
2715	F	63	137	248	Killed	—	—	—	—	—	—	—	—	—
2716	F	58	137	248	Do	—	—	—	—	—	—	—	—	—

2717	M	54	179	248	Do	—	—	—	—	—	—	—	—	—
2718	M	59	200	248	Do	—	—	—	—	—	—	—	—	—
2719	M	60	60	74	G U Disease, ascitis	—	—	—	—	—	—	—	+++	208·0
2720	M	75			Used for biochemical studies									
2721	M	68			Do									
2722	M	75	59	66	Pneumonia	—	—	—	—	—	—	—	—	—
2723	M	74	209	248	Killed	—	—	—	—	—	—	—	++	96·0
2724	M	70	168	248	Do	—	—	—	—	—	—	—	—	—
2725	M	66	202	248	Do	—	—	—	—	—	—	—	+++	340·0
2726	M	71	204	248	Do	—	—	—	—	—	—	—	—	—
2727	M	60	164	248	Do	—	—	—	—	—	—	—	—	—
2728	M	63	222	248	Do	—	—	—	—	—	—	—	—	—
Totals		1,491	3,258	4,346		1	0	0	1	0	0	0	4	
Averages		64·8	138·9	207 0									·	

In No 2719 the bladder was ruptured owing to great distension, urine and lime in abdominal cavity

In Nos 2708, 2723 and 2725 the bladder stones were well formed and numbered 2, 3 and 6 respectively; Nos 2723 and 2725 were calcium carbonate stones, No 2708 was not analysed, in No 2719 the concretions were of the usual gravelly character, noted in previous tables, they were composed of calcium hydroxide

TABLE VIII

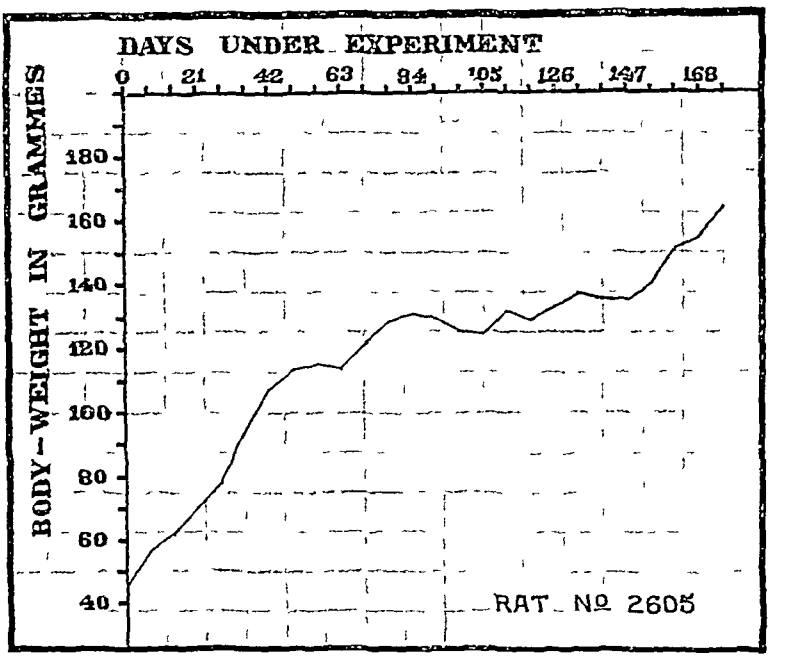
Being a summary of Tables I to VII

Table number	Diet	Principal season in which experiment was carried out	Average number of days of life	Cystitis	Pyonephrosis	Hydronephrosis	Dilated ureters	Stone in ureters	Stone in kidney	Stone-in-the-bladder	Average weight of stones in kidney mg	Average weight of stones in bladder mg
I	White bread and yeast	Summer	78·0	2						5		10 *
II	White bread and yeast	Winter	139·5	4		1	2	1		2		31 *
III	White bread, yeast and lime	Summer	122·4	11	4	2	9	1	3	10	8 *	12 *
IV	White bread, yeast and lime (3 grains)	Winter	106·9	14	6	3	12	3	4	17	531	361
V	White bread, yeast and lime (3 > 1·5 grains)	Winter	76·2	12	6	2	10	3	3	12	16	51
VI	White bread, yeast, lime (3 > 1·5 grains) and gingelly oil	Winter	72·7	17	11	3	16	7	7	18	33	67
VII	White bread, yeast, lime (3 > 1·5 grains), gingelly oil and radiostoleum		207·0	1	0	0	1	0	0	4		187

* Approximate average weight

former provided insufficient material for complete qualitative analysis, the latter was found to consist of ammonium-magnesium-phosphate (Table IX)

CHART 1



Showing the growth-curve of rat No 2605 (Table II) fed on the basal diet of white bread, yeast and distilled water

Influence of lime

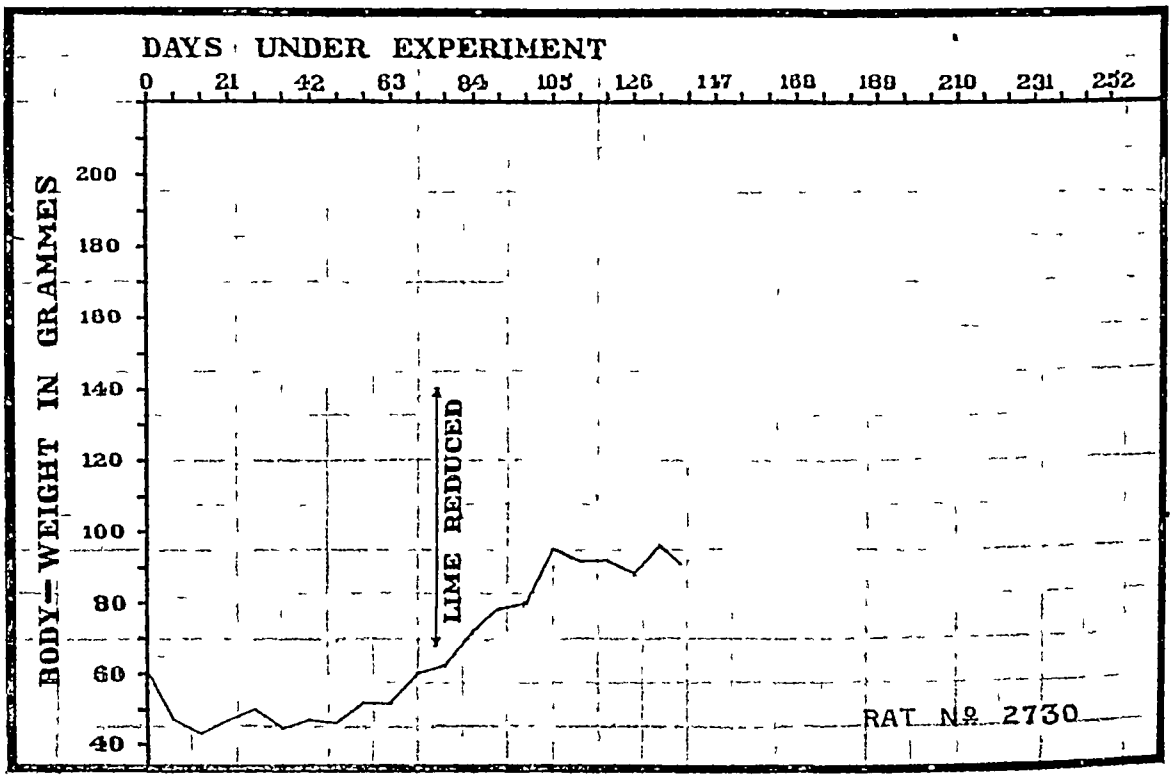
The addition of lime to the basal diet had four notable effects (*a*) growth was impaired (Chart 2), (*b*) the incidence of calculus disease was greatly increased, (*c*) the incidence of urinary tract infections was likewise greatly increased, (*d*) the physical and chemical characters of the calculous deposits were altered (Table IX) The incidence of vesical calculus (Table VIII) rose from 14 6 to 56 2 per cent, that of renal calculus from 0 to 14 6 per cent, and that of ureteral calculus from 2 to 8 per cent The incidence of cystitis rose from 12 5 to 52 0 per cent, that of pyonephrosis from 0 to 21 per cent, that of hydronephrosis from 2 to 10 per cent, and that of dilated ureters from 4 to 44 per cent

Characters and composition of calculous deposits

The stones occurring in rats fed on the basal diet without added lime were composed mainly of ammonium-magnesium-phosphate and had the physical characters previously described (McCarrison, *a*, *b*, *c*, 1928, and 1930*a*) Those occurring in rats, to whose basal diet lime was added, were of very different character and composition The 'calculous deposits'—a more fitting description than 'stone'—were then made up of numerous, smaller or larger, rounded

and non-facetted grains or pellets of chalk-like, gravelly material, whitish in colour and non-pigmented (Plate LI, fig 1) In quantity they varied greatly in different cases In some the deposits weighed only a few milligrams, in others they were present in enormous quantities, the maximum amount being 2,085 milligrams or one-seventieth part of the body-weight of the animal (No 2641, Table IV) The deposits in the bladder ranged in weight from a few milligrams to 1,250 mg , those in the kidney from a few milligrams to 1,530 mg (Plate LI, fig 1), while those in the ureter existed only as small,

CHART 2



Showing the growth-curve of rat No 2730 (Table V) fed on the basal diet *plus* lime Compare with Chart 1 and note the inhibition of growth caused by the high intake of lime during the first 75 days of the experiment and the improvement in growth after the dose of lime was reduced

non-impacted grains Strictly speaking the last cannot be regarded as ureteral stones at all but merely as fragments of mineral matter on their way from the kidney to the bladder, in a few cases, however, these fragments were large enough to permit of the term 'stone' being applied to them, for purposes of record all cases in which calculous deposits were found in the ureter have been classed as 'ureteral calculi'

Four types of calculous deposits were met with in these experiments (*a*) ammonium-magnesium-phosphate stones, (*b*) calcium carbonate stones, (*c*) calcium hydroxide stones, and (*d*) stones made up of a mixture of the last two substances The first existed only in rats to whose basal diet lime

was not added, the last three only in rats to whose basal diet lime was added Their composition has been dealt with in previous papers (Newcomb and Ranganathan, 1930, Ranganathan, 1930) For the sake of completeness their average composition is given in Table IX

TABLE IX

Showing the average composition of the four types of calculous deposits met with in these experiments

	Type of stone	Moisture Per cent	As percentages on moisture-free samples				
			Total nitrogen	P_2O_5	CaO	MgO	CO_2
1	Ammonium-magnesium-phosphate stones	37·8	8·3	33·1	1·1	13·8	
2	Calcium carbonate stones	8·0	1·36	1·38	41·74	1·8	38·8
3	Calcium hydroxide stones	26·45	1·45	1·31	31·86	0·2	
4	Mixed calcium carbonate and hydroxide stones	12·05	1·50	0·69	36·32	0·97	17·86

Influence of season.

The quantity of lime deposited in the urinary tract was very much greater during the winter (Table IV) than during the summer months (Table III) in rats to whose basal diet lime was added The incidence of calculous disease was also higher in winter, as was that of cystitis, pyonephrosis and dilated ureters (Table VIII) Rats to whose diet lime was not added tended to live longer and to suffer less from infectious disease during the winter than during the summer (Tables I and II)

Influence of gingelly oil

The addition of gingelly oil to the diet of white bread, yeast and lime caused a slight improvement in growth (Chart 3), but a significant increase in the incidence of calculous disease (Tables V and VI) More lime was deposited in the urinary tract, and inflammatory states of the tract—cystitis, pyonephrosis —were more frequent and more severe

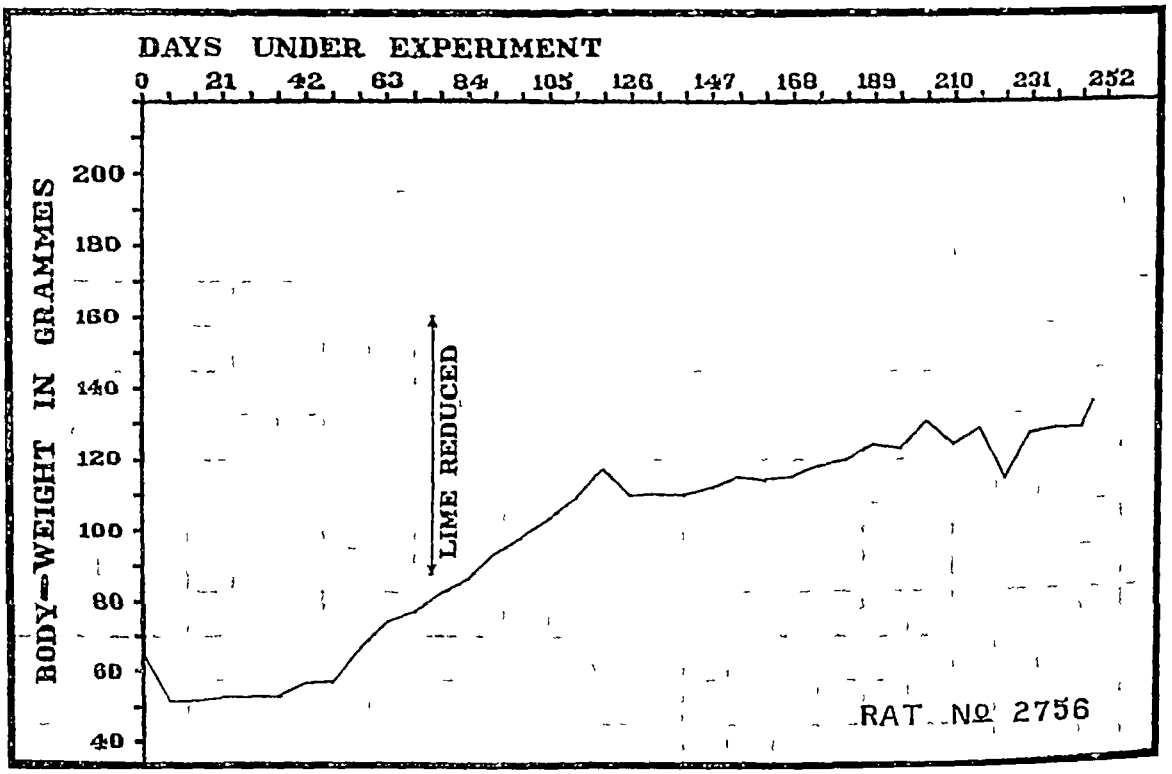
Influence of radiostoleum

The further addition of radiostoleum to the diet of white bread, yeast, gingelly oil, lime and distilled water had very striking effects (Tables VI, VII and VIII) The mortality was greatly reduced, the incidence of calculous disease and of inflammatory states of the urinary tract was markedly lowered,

and, pressure effects were almost wholly absent Thus, the incidence of vesical calculus fell from 81 8 to 19 0 per cent, of renal calculus from 31 8 to 0 per cent, and of ureteral calculus from 31 8 to 0 per cent That of cystitis fell from 77 2 to 4 8 per cent and that of pyonephrosis from 50 to 0 per cent Hydronephrosis did not occur at all and dilated ureters occurred only in one case, a drop in the incidence of the latter of from 72 5 to 4 5 per cent

The experiments (Tables V to VII) with which these results deal were divided into two periods that of high lime-ingestion from the first to the 75th day, and that of lower lime-ingestion from the 75th to the 248th day During the first period the protection afforded by the radiostoleum against

CHART 3



Showing the growth-curve of rat No 2756 (Table VI) fed on the basal diet *plus* gingelly oil and lime Compare with Chart 1 and note the inhibition of growth produced by the lime

calculous disease was considerably less than during the second period, indeed, it would seem that during the latter period it was complete and that calculous deposits formed during the first period were resolved either wholly or in part Three animals died during the first period (Table VII), of these one (No 2708) had calculous deposits in the bladder of the same physical and chemical characters as those met with in animals not receiving radiostoleum (Plate LI, fig 1) During the first 12 days of the second period two more animals died and of these one (No 2719) had similar calculous deposits in the

bladder, which were obviously the residual effects of the first period of high lime-ingestion In this case, however, the calculous deposits were well formed and existed as 2 large, softish stones Thus 2 out of 5 rats—or 40 per cent—had calculous deposits in the bladder despite the ingestion of radiostoleum It is reasonable, therefore, to assume that five or six of the remaining 16 had concretions in the urinary tract at the time the dose of lime was reduced But on killing these 16 animals on the 248th day of the experiment two only (Nos 2723 and 2725) were found to have vesical calculi It would seem, therefore, that new cases of stone did not arise after the 75th day and that spontaneous cure of stones already formed by that time had occurred in some cases Evidence highly suggestive of such spontaneous cure is afforded by the two cases of vesical calculus found amongst these 16 animals Nos 2723 and 2725 (Table VII) The physical characters of these calculi (Plate LI, fig 3) differed strikingly from those of the calculous deposits found in other animals (Plate LI, fig 1) They were well formed, there being three stones in one case (No 2723) and six in the other (No 2725) They were facetted and dark in colour, resembling gall-stones, on exposure to air their colour faded to a lighter hue On breaking them they were found to consist of a brittle shell enclosing a dirty-white, coarse, spongy material, resembling pumice stone The spongy material within the brittle shells was strongly suggestive of resorption of part of the calculous material deposited during the first period of the experiment, while its enclosure in the shell conveyed the impression of a protective encystment of the non-absorbed calculous material The shell was certainly deposited around this material during the second period of the experiment On analysis of these stones the shell, as well as its contents, was found to be made up of calcium carbonate with a little magnesium and phosphate They contained less moisture, less nitrogen, and less phosphates than the calcium carbonate deposits occurring in other cases (Table IX), due, possibly, to the fact that the latter were not washed free of adherent urine before analysis

Their composition is given in Table X

TABLE X

Giving the composition of the stones occurring in two rats (Nos 2723 and 2725, Table VII) receiving radiostoleum

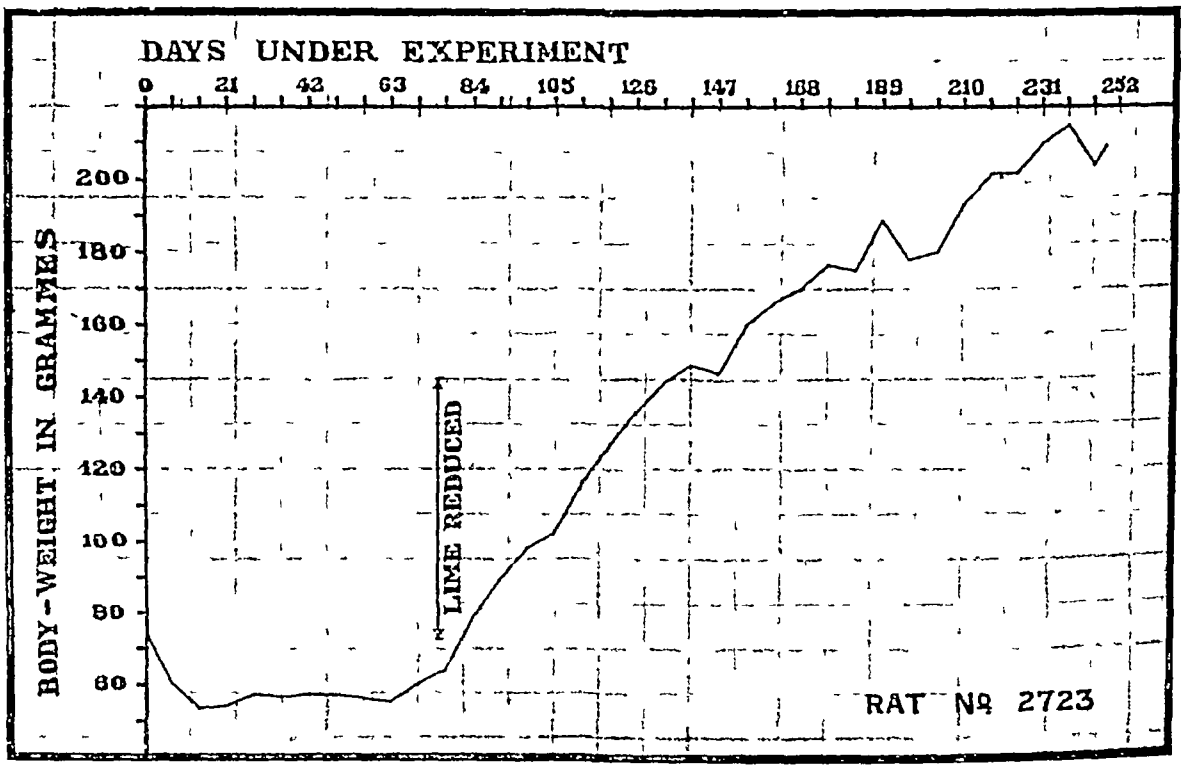
Rat number	Moisture Per cent	As percentages on moisture-free sample						Murexide test
		Total nitrogen	P_2O	CaO	MgO	CO_2	C_2O_2	
2723	2·78	0·58	1·12	48·8	2·2	38·0		Negative
2725 *	3·21	0·44	0·85	48·9	1·8	41·4		Negative
2725 †	2·60	0·75	1·00	48·9	1·9	39·4		Negative

* Represents an average sample of stone from rat No 2725

† Represents only the outer shell of the stone from rat No 2725

The rats in which these calculi occurred were well nourished, even fat then bladder walls were hypertrophied and the mucous membrane hyperplastic, but in neither case was cystitis, nor other inflammatory state of the urinary tract, present The hypertrophic changes in the bladder represented the healthy response of well-nourished tissues to undue stimulation caused by a foreign body in the viscus, contrasting in this regard with the degenerative, metaplastic and inflammatory changes present in the tissues of the urinary tract of rats not receiving radiostoleum

CHART 4

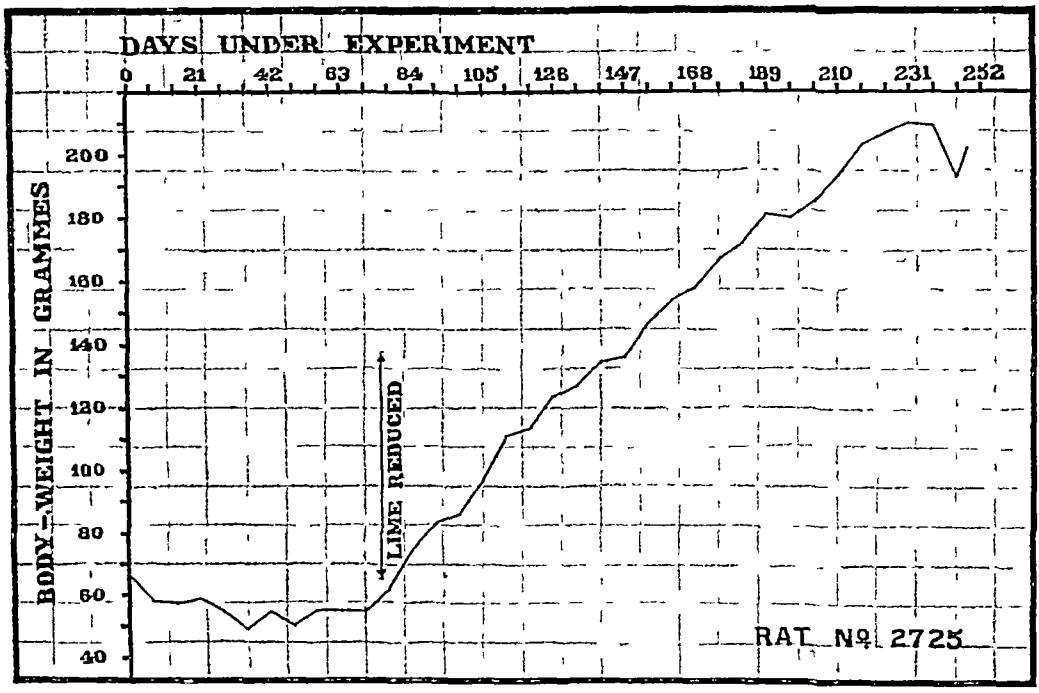


Showing the growth-curve of rat No 2723 (Table VII) in whose bladder two calcium carbonate stones were present Rat fed on the basal diet to which gingelly oil, lime and radiostoleum were added The animal was otherwise healthy Note inhibition of growth caused by the lime during the first 75 days of the experiment, and the good growth after the concentration of lime in the diet was halved

I conclude from these observations that while radiostoleum had a pronounced effect in counteracting the stone-producing influence of the lime, the amount ingested by the rats during the first period of the experiment was deficient relative to the amount of lime ingested The deposition of lime in the urinary tract was not, therefore, wholly prevented, though the number of rats affected in this way was significantly less than in those not receiving radiostoleum During the second period the amounts of lime and radiostoleum were more evenly balanced, fresh deposits of lime did not then occur in the kidney while those already deposited in the bladder tended to be resolved or to

be surrounded by a brittle coating of calcium carbonate The unresolved remnants of previous deposits became, in fact, foreign bodies around which urinary salts were laid and since these salts were mostly made up of calcium carbonate (Table XI) the coating of the unresolved remnants of previous deposits was composed mainly of this material This interpretation of events is supported by the differing effects of radiostoleum on growth during the two periods of the experiment During the first period, when the ingestion of lime was high, growth in the majority of animals was almost at a standstill (Charts 4 and 5), while in a minority (Chart 6) the inhibition of growth was less

CHART 5

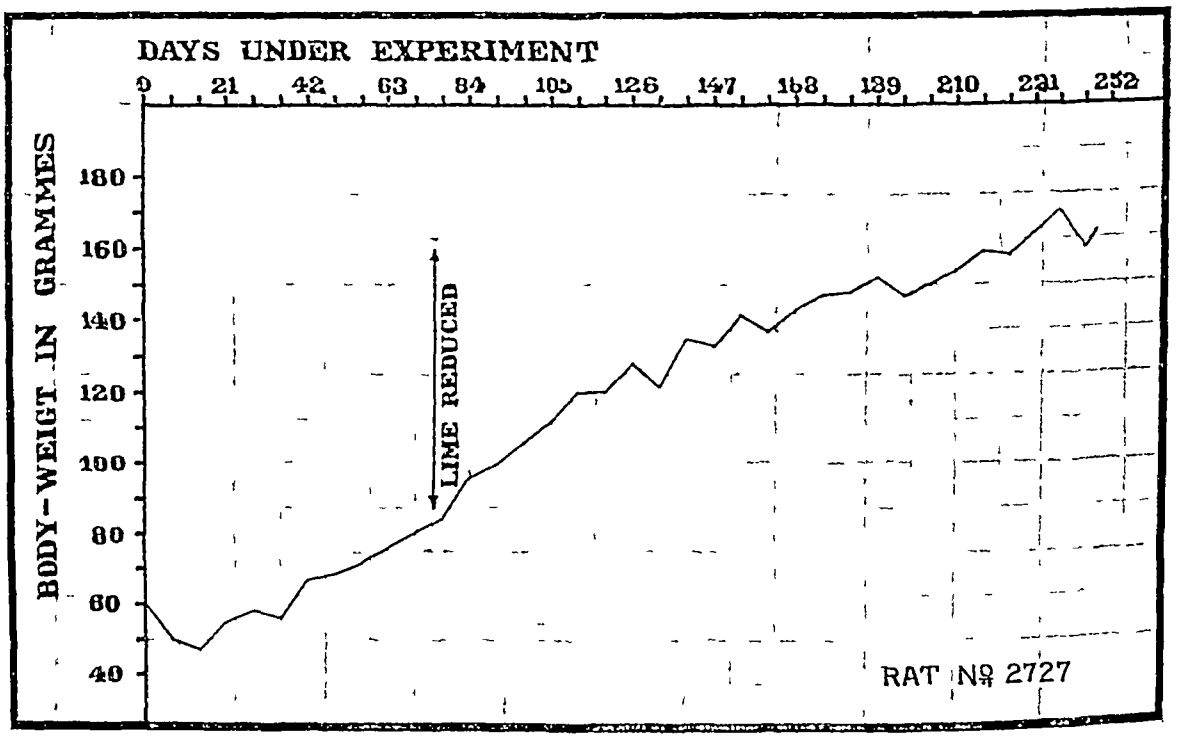


Showing the growth-curve of rat No 2725 (Table VII) in whose bladder six calcium carbonate stones (Plate LI, fig 3) were present Rat fed on the basal diet to which gingelly oil, lime and radiostoleum were added The animal was otherwise healthy Note inhibition of growth caused by the lime during the first 75 days of the experiment, and the good growth after the concentration of lime in the diet was halved

marked During the second period, when the dose of lime was reduced, growth proceeded normally This difference in growth was due in considerable part to a higher food-consumption during the second period, but it seems also to have been due to an inhibitory action of the high lime-ingestion on the growth-promoting properties of the radiostoleum However this may be, a balanced adjustment of these two ingredients of the experimental diet was found to be necessary not only for normal growth but for the prevention of calculous disease and of inflammatory states of the urinary tract

A peculiar interest attaches to these two cases of stone they were obviously the outcome of a period of malnutrition followed by one of more perfect nutrition but in which the food was still faulty with respect to calcium-phosphorus balance Such a train of events is not unlikely to occur both in human beings and in animals afflicted by 'stone' periods of want alternating with periods of plenty The sequence of events in the formation of these calculi suggests that both periods may play a part in stone-formation the one when concretions are actually deposited in the urinary tract, the other when 'excess' of certain mineral substances in the urine may add to the stones' architecture The laminated character of many stones may have some such explanation But the chief interest in these two stones is that for the first

CHART 6



Showing the growth-curve of rat No 2727 (Table VII) This animal was fed on the basal diet to which gingelly oil, lime and radiostoleum were added It did not suffer from stone Note that the inhibition of growth during the first 75 days of the experiment, when the concentration of lime in the diet was high, was less marked than that shown in Charts 4 and 5 This result was exceptional

time, in the course of our work on this subject, vesical calculus has been found in otherwise healthy and well-nourished rats Always, in previous experimental work, the condition has occurred in animals suffering not only from stone but from other effects of vitamin-deprivation In these two cases the sole abnormality was the presence of calculi in an hypertrophied but otherwise healthy bladder The significance of this observation is, therefore, great

Relation of radiostoleum to calcium and phosphate excretion.

Six days after the reduction in the dosage of lime two rats from each of the three groups (Tables V VI and VII) were placed in metabolism cages and weekly estimations made of their intake and urinary excretion of calcium and phosphate They were observed in this way for a period of six weeks For purposes of comparison four well-fed control rats were examined in a similar way Two of these had no lime added to their stock diet the other two had the lime being added in the same proportion as in the experimental diets (1·5 grains per rat per day) The results of these observations made by my assistant Mr S Ranganatran are as follows —

	Diet	Calcium (CaO)			Phosphates (P O)		
		Intake	Excreted through the kidneys	Percentage excreted through the kidneys	Intake	Excreted through the kidneys	Percentage excreted through the kidneys
		mg	mg		mg	mg	
1	Stock	5436	449	5·31	10283	4407	42·80
2	Stock and lime	21373	465	2·18	10252	1622	15·84
3	White bread, yeast and lime	9391	1390	15·80	2866	87	3·04
4	White bread, yeast lime and gingelly oil	11525	1712	14·90	3200	122	3·89
5	White bread, yeast lime gingelly oil and radiostoleum	13143	3203	24·50	4107	124	3·02

From these results it is seen that in well-fed rats there was no increased excretion of calcium through the urinary tract consequent on the addition of lime to the stock diet There was however a considerable decrease in the urinary excretion of phosphates due to the increased demands of the faecal calcium (an assumption confirmed by the close parallelism which was found to exist in these animals between the faecal excretion of calcium and phosphates*).

When the same amount of lime was added to the phosphate-poor basal diet the urinary excretion of calcium was very high while that of phosphates was very low Consequent on the excessive ingestion of lime the animals were robbed of much of the small amount of phosphorus which the basal diet contained The food did not contain even enough phosphorus to combine with

*Further details in this regard are reserved for a subsequent paper

the fæcal calcium The presence or absence of gingelly oil in the experimental diet had no effect on the course of the lime and phosphate excretion

With the further addition of radiostoleum there was an even greater excretion of lime through the kidney, due in part at least to an increased intake, but the urinary excretion of phosphates was not altered

Mode of action of radiostoleum.

It follows from the above observations that the tendency of radiostoleum to inhibit the deposition of lime in the urinary tract was not due to its limitation of the amount of lime passing through the kidneys, the limiting factor was phosphorus not radiostoleum Whether it prevented the deposition of lime solely by maintaining the nutrition and functional integrity of the tissues of the urinary tract itself, and then freedom from infection, or by an alteration in the physical state of the calcium salts passing through the kidneys, remains to be determined

Incrusted cystitis.

It will be observed from Tables I, II, III and IV that 8 cases of incrusted cystitis occurred in this series of experiments So far as I am aware the experimental production of this unusual condition has not previously been reported It occurred in rats to whose diet lime was added as well as in others to whose diets lime was *not added, but no case* was observed in animals whose diets contained gingelly oil with or without radiostoleum The latter observation may not be without ætiological significance

Similarity of cattle-stones to calcium carbonate stones in rats

In a succeeding paper (Ranganathan, 1931) (page 935) Mr S Ranganathan gives an account of his analyses of cattle-stones Their composition, as well as their physical characters (Plate LI, fig 4), approximates closely to that of the calcium carbonate stones (Plate LI, figs 1 and 3) produced in these experiments In making this comparison count is taken only of those rat-stones that are composed for the most part of calcium carbonate and not of those containing this substance in admixture with calcium hydroxide This similarity of composition is shown in Table XI

It will be observed from Table XI that the two stones occurring in rats fed on a diet containing radiostoleum (Nos 2723 and 2725) are practically identical in their composition with that of cattle-stones, the only difference between them being that the cattle-stones contain twice as much magnesium This observation may have an important bearing on the ætiology of 'stone' in cattle

Summary.

1 Well-fed albino rats living under hygienic conditions of life are normally free from stone, they develop the disease only when fed on deficient diets

TABLE XI

Showing the composition of cattle-stones and of the calcium carbonate stones of rats

Average	Moisture Per cent	As percentages on moisture-free samples				
		Total nitrogen	P_2O_5	CaO	MgO	CO_2
Cattle-stones (23)	3·1	0·4	0·9	44·0	4·8	39·1
Rat-stones (20)	8·0	1·36	1·38	41·7	1·8	38·8
Rat-stones Nos 2723 and 2725	3·0	0·5	1·0	48·9	2·0	39·7

2 The dietetic factors so far found to be concerned in stone-production in albino rats are of two orders negative and positive The negative factors are deficiency of fat-soluble vitamins and deficiency of phosphates, the positive factors are excess of lime and an unknown agent present in varying amounts in cereal grains The positive factors appear to be innocuous in the presence of a sufficiency of the negative ones

3 Malnutrition of the urinary tract was an important underlying cause of the calculous disease occurring in rats in these experiments Superimposed infection may have contributed to its occurrence, it was not essential to it In a number of cases cystitis and pyonephrosis occurred without stone, in a number of others stone occurred without cystitis or pyonephrosis Incrusted cystitis was observed in 8 cases

4 Although an excess of vitamin D in the diet is known to cause stone in albino rats, the daily exposure of these animals to the direct rays of the sun did not increase the incidence of the disease when they were fed on a stone-producing diet

5 The incidence of urinary calculus in rats fed on a basal diet of white bread and yeast was 14 6 per cent The calculi so produced were of the ammonium-magnesium-phosphate variety

6 The addition of slaked lime to the basal diet of white bread and yeast greatly increased the incidence of urinary calculus and of urinary tract infections It also altered the physical and chemical characters of the calculi, which were no longer well formed nor composed of ammonium-magnesium-phosphate but were ill formed and composed of calcium carbonate or calcium hydroxide or both More lime was deposited in the urinary tract during the winter than during the summer months

7 The stone-producing potency of this diet (white bread, yeast and lime) was further enhanced by the addition to it of a vitamin-poor vegetable oil, inflammatory states of the urinary tract were also more frequent and more

severe, due probably to the greater relative deficiency of fat-soluble vitamins brought about by the presence of this oil in the diet

8 The further addition of radiostoleum (approximately 0 06 of a drop per rat per day) to this diet caused a great reduction in the incidence of urinary calculus and of urinary tract infections The stone-preventing potency of this amount of radiostoleum appeared to be related to the concentration of lime in the diet the more lime the less potent the radiostoleum

9 Radiostoleum did not prevent the deposition of lime in the urinary tract by limiting the amount of lime absorbed or passing through the kidneys Whether it prevented the deposition of lime solely by maintaining the nutrition and functional integrity of the tissues of the tract and their freedom from infection, or by altering the physical state of the calcium salts passing through the tract, remains to be determined

10 A balanced adjustment between the lime and the radiostoleum was found to be necessary not only for the prevention of calculous disease but for normal growth of the rats

11 Vesical calculus was produced in otherwise healthy and well-nourished rats by feeding them for 75 days on a diet of high stone-producing potency and thereafter for 173 days on a diet of which the stone-producing potency was low or nil

12 Experimentally-produced calcium carbonate stones in rats have practically the same composition as urinary calculi in cattle, an observation which may have an important bearing on the ætiology of cattle-stone

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PLATE LI

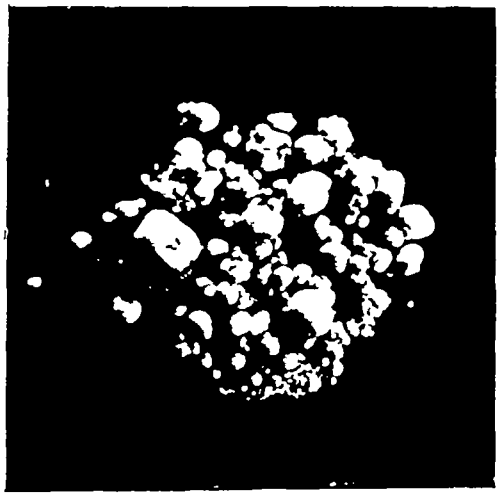

Fig 1

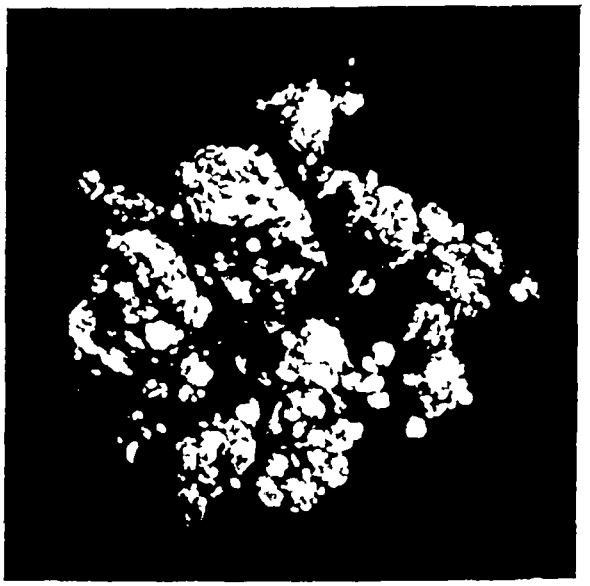

Fig 2

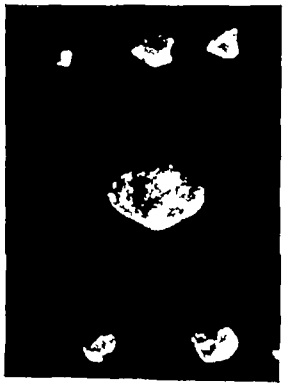

Fig 3

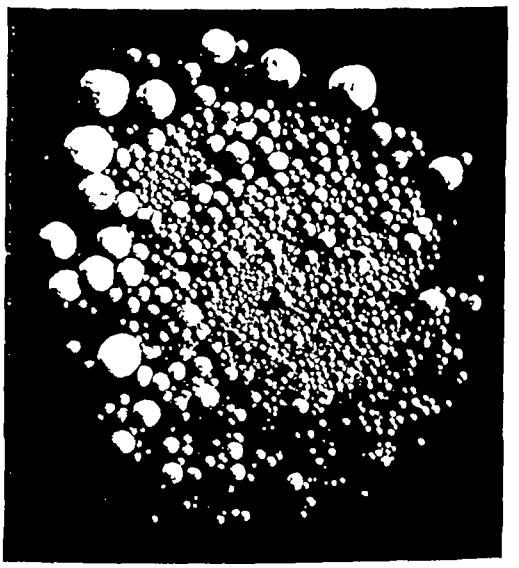

Fig 4

Explanation of Plate LI

Fig 1 Showing calcium carbonate stones, weighing 715 mg, removed from the bladder of rat No 2618 (Table IV) Compare with the 'stones' removed from a bullock (Fig 4)

„ 2 Showing calcium carbonate concretions, weighing 1,530 mg, removed from the kidneys of rat No 2641 (Table IV)

„ 3 Showing six calcium carbonate stones removed from the bladder of an otherwise healthy rat (No 2725, Table VII) fed on the basal diet to which gingelly oil, lime and radiostoleum were added For full description *see* text

„ 4 Showing 'stones' removed from the urinary passage of a bullock The stones are composed of calcium carbonate Compare with the experimentally-produced calcium carbonate stones in rats (Fig 1)

CHEMICAL COMPOSITION OF URINARY CALCULI IN CATTLE

BY

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THE present paper deals with twenty-three urinary calculi obtained from cattle, seventeen of which were from various parts of the Madras Presidency, four from Nadiad and Barsi in the Bombay Presidency, and two from Jaiwal and Fatehpur in the United Provinces Twenty-one were vesical and urethral stones and two were renal Particulars in regard to them are given in Table I

Physical characters of the calculi

Cattle stones are usually small, grain-like bodies, often little bigger than a No 4 lead-shot, having a light or deep golden yellow sheen about them Most of them look alike in structure and shape, this is true of only the vesical and urethral stones The two renal stones stand apart from the rest as regards their physical characteristics they are of irregular shape, the shape presumably depending on the site of formation of the calculus in the kidney, and devoid of the bright metallic sheen

The cattle stones differed in their physical characteristics from human calculi in the following regards —

(1) They are much smaller, rarely exceeding the size of a small pea, whereas human stones are commonly of large size

(2) They are usually multiple, often occurring to the number of 100 or more less often they are few in number Human stones, on the other hand, are commonly single, less often they are multiple

(3) Cattle stones have a laminated structure of several layers, all looking alike, the layers being thin and comparable to the thin coats of the onion While the different layers in the human stones have a different composition, consisting either of calcium oxalate, or uric acid or urate, or calcium or

TABLE I

Giving particulars as to age, food, etc., of cattle suffering from urinary calculus

Stone number	Animal	Age in years	District	Location of stone	Food eaten by animal	Extent and nature of available grazing
1	Bullock	4	Cuddapah	Urethra	32 lb fodder (dry and natural) such as cholam (*Andropogon Sorghum*), koria (*Cyperus*), ragi (*Eleusine Coracana*) per day, gram powder and Bengal Pottu (*Cicer Arietinum*) about 1 lb at a time	Nil
2	Bullock	6	Cuddapah	Urethra	Dry fodder stalks of cholam, ragi and paddy (*Oryza Sativa*) about 21 to 32 lb per day Crushed gram such as cholam 3 lb of powdered gram and cotton-seed (*Gossypium Sp*) per day	Nil
3	Bull	6	Cuddapah	Urethra	32 to 40 lb of dry fodder (cholam, ragi and paddy straw), 12 lb of cotton-seed, powdered gram, cholam and ragi	Nil
4	Bullock	10	Kistna	Bladder and urethra	Horse gram (*Dolichos Biflorus*), rice bran, gingelli cake and green maize (*Zea Mays*) Roughage green maize plants, paddy straw, green grass and hemp plants (*Hibiscus cannabinus*)	++
5	Bullock	12	Guntur	Kidneys, bladder and urethra	Straw, sun-hemp (*Crotalaria juncea*), horse gram during the working season and green grass in winter	Nil
6	Bullock	Adult	Kurnool	Urethra	Cotton-seeds, kulthi (*Dolichos Biflorus*), cholam and ground-nut fodder (*Arachis hypogea*)	Nil
7	Bull	Adult	Kurnool	Bladder and urethra	Cholam stalks, dry or green *ad libitum* and dried ground-nut plants, crushed kulthi only during working season, crushed and soaked cotton-seed (whole) only in working season	Nil
8	Bull	Adult	Kurnool	Urethra	Cholam and ground-nut fodder, cotton-seed crushed and kulthi crushed in the working season only	Nil
9	Bull-calf	0 5	Kurnool	Bladder	Mother's milk, cholam stalks, green grass, cotton-seed and rice water	Nil
10	Bull	Adult	Kurnool	Urethra	Cotton-seed, cholam and ground-nut fodder	
11	Bullock	8	Tanjore	Urethra	3 lb cotton-seeds, 2 lb horse gram and 20 lb straw per day	Nil

12	Bullock	6	Kurnool	Urethra	Dry jarry grass for 4 months, green grass for 4 months, green jarry grass for 4 months *ad libitum*, cotton-seeds, korra (*Setaria Italica*) and horse gram	
13	Bullock	8	Kurnool	Urethra	Green fodder *ad libitum*, crushed horse gram and cotton-seeds, husks of gram and ground-nut, 2 lb boiled horse gram and 8 lb of cotton-seeds per day	
14	Bullock	8	Kistna	Urethra	2 lb boiled horse gram, 3 lb rice bran and 2 lb of cotton-seed, dried cholam stalks as fodder	Nil
15	Collection of stones from several animals		Kistna		History sheets not available	
16			Kistna		History sheets not available	
18	Bullock	7	S Arcot	Urethra	6 lb bran, 2 lb oil cakes and 40 lb straw per day	Nil
19	Bullock	8	Nadiad, Kaira Dt (Bombay Presidency)	Urethra	Jowari (*Sorghum Vulgare*), bajri (*Pennisitum Typhoidium*) and gowar (*Cyamopsis psoralrides*)	Nil
20	Bullock	10	Nadiad, Kaira Dt (Bombay Presidency)	Urethra	Jowari, bajri and gowar	Nil
21	Bullock		Barsi	Urethra	Karbi (jowari stalks) for 8 months of the year, green grass during 4 months of the rainy season, cotton-seeds and oil cakes	
22	Bullock		Barsi	Urethra	Karbi (jowari stalks) for 8 months of the year, green grass during 4 months of the rainy season, cotton-seeds and oil cakes	..
23			Jarwal, Bahraich, U P	Pelvis of kidney		Little grazing in the jungles
24	Ox		Fatehpur, U P	Right kidney	Bran and husk of wheat (*Triticum Sp*)	

TABLE II

Stone number	Moisture Per cent	As percentages on moisture-free samples										
		Total nitrogen	P_2O_5	CaO	MgO	CO_2	C_2O_2	Ash	Insoluble ash	Ash not accounted for	Murexide test	Total 4 + 5 + 6 + 7 + 8 + 10 Per cent
1	2·1	0·2	Nil	46·6	6·2	40·2		53·8	0	1·0		93·0
2	1·7	Nil	Nil	60·8	Nil	36·7		61·1	0	0·3		97·5
3	2·4	Trace	Trace	46·5	5·7	38·8		53·8	0	1·6		91·0
4	3·5	0·4	Trace	44·4	9·4	28·4 (?)		54·2	0	0·4	0	82·2 (?)
5	3·0	0·5	1·7	42·3	8·1	39·0		53·7	0	1·6	0	91·1
6	2·6	0·2	Trace	46·7	5·8	39·2		53·6	0	1·1	0	91·7
7	2·3	0·3	1·0	47·3	5·7	38·5		53·4	0	—0·6	0	92·5
8	2·3	0·5	0·8	45·5	2·4	39·9	1·1		0		0	89·7
9	1·9	0·6	1·0	46·8	3·1	40·5	0·8		0		0	92·2
10	4·1	0·4	1·0	42·5	9·3	38·0		52·8	0	1·0	0	90·8
11	3·5	0·7	0·9	44·3	4·5	40·8	0·5		0		0	91·0
12	4·1	0·3	Trace	48·5	2·6	40·8		53·9	0	2·8		91·9
13	4·4	0·7	1·1	38·7	3·4	41·5	0·6		0		0	85·3
14	5·0	0·9	1·3	36·8	4·4	39·6	1·3		0		0	83·4
15	1·8	0·4	0·7	45·2	2·2	41·7	0·9		0		0	90·7
16	2·1	0·5	0·8	45·0	3·5	39·5	0·7		0		0	89·5
18	3·5	Trace	1·2	41·2	4·0	39·3	0		0		0	85·7
19	4·5	0·2	1·7	40·1	5·3	42·0			0		0	89·1
20	4·0	0·3	1·5	40·6	4·6	40·2			0		0	86·9
21	4·2	0·3	1·9	39·8	6·8	37·5			0		0	86·0
22	2·2	0·3	1·2	41·5	4·8	40·7			0		0	88·2
23	3·0	0·3	1·6	44·5	3·4	41·0			0		0	90·5
24	3·4	0·5	1·2	36·7	6·1	34·7			14·0		0	92·7

magnesium ammonium phosphate, those of cattle stones consist for the most part of calcium carbonate Again, the several layers of the human stones are differently coloured—the colours serving as an approximate guide to the chemical composition, e g , uric acid or urate layers being light yellow to dark brown, phosphate layers white to dirty white, and oxalate layers being chocolate-coloured—whereas those of cattle stones have nearly the same colour

(4) Cattle stones are quite hard, considerable pressure being usually necessary to powder them Human stones may also be hard if they consist mostly of calcium oxalate, comparatively less hard if consisting of uric acid or urate, and relatively brittle if of phosphates

(5) Cattle stones have all nearly the same colour, which ranges from whitish-yellow to yellowish-brown, commonly they have a metallic sheen Human stones do not all have the same colour nor have they the bright metallic sheen, so common in cattle stones

(6) Whereas all cattle stones resemble one another closely in most of their physical characters, human stones do not

Chemical composition of cattle stones.

The similarity of cattle stones in most of their physical characters is manifested in their chemical composition as well (Table II)

Methods of analysis—Nine of the twenty-three stones were of sufficient size to permit of a quantitative macro-analysis moisture, total ash, ash insoluble in hydrochloric acid, total nitrogen, phosphates, calcium and magnesium were thereby determined The same ingredients, with the exception of the total ash, were determined in the remaining fourteen by the micro-methods developed in this laboratory Oxalates and carbonates were determined by micro-methods in all the twenty-three stones A qualitative test (Murexide test) for the presence of uric acid was done in all of them The results of the chemical analyses are given in Table II and the average composition of the stones in Table III

TABLE III

Average composition of the stones

Total ash		Per cent
P_2O_5	0·90	54·5
CaO	44·0	
MgO	$\frac{4·84}{49·74}$	
C_2O_3		0·59
N		0·37
CO_2		39·07

The percentage of ash, not accounted for in the nine stones wherein total ash was estimated, is given in column 11 of Table II, its average being 1 02 per cent

It is apparent from Table II that all the calculi have a similar composition, consisting mostly of carbonate of calcium, with a little magnesium The magnesium content varied from 2 2 to 9 4 per cent with an average of 4 84 per cent, presumably it exists for the most part as carbonate, though it is possible that some may exist in the form of phosphate The average percentage of CaO being 44 0, it would require only 34 5 per cent of CO_2 to form $CaCO_3$, while there actually exists 39 07 per cent of CO_2 The remaining 4 57 per cent of CO_2 is presumably combined with magnesium to form magnesium carbonate, if that were so, it would require only 1 19 per cent of MgO whereas the average for MgO works out to 4 84 per cent The balance of 0 65 per cent MgO is very likely united with the P_2O_5, the average content of which is 0 90 per cent

Nitrogen is present in cattle stones only in traces which rarely exceed 0 5 per cent, the average being 0 37 per cent The Murexide test shows that the nitrogen does not exist as uric acid, the nature of the nitrogenous compound was not, however, investigated, as it occurred in exceedingly small amounts and as there was not a sufficiency of stone material to admit of the determination of its nature Excluding the values obtained for nitrogen, the total constituents—expressed in column 13 (Table II) as the sum of P_2O_5, CaO, MgO, CO_2, C_2O_3 and insoluble matter—amounted to 89 7 per cent of the dry weight of stones Assuming that the nitrogen exists as a protein—this assumption is warranted by the experience gained in the analysis of human vesical calculi—the average for the total would be raised to 92 0 per cent of the dry weight of the stone, still leaving 8 0 per cent not accounted for

Insoluble matter—i e, portions of the stone left undissolved after digestion with concentrated sulphuric acid—occurred only once, in stone No 24, which happened to be a kidney stone removed from an ox The only other kidney stone (stone No 23) did not contain any insoluble matter

The chemical composition of the only two renal stones encountered was not significantly different from that of the rest In this respect, cattle approximate closely to rats, whose renal stones do not differ in their chemical composition from their vesical ones (Newcomb and Ranganathan, 1930, Ranganathan, 1930), and differ from human beings in whom kidney and bladder stones are essentially different in their composition (Newcomb and Ranganathan, 1930*b*)

From a study of Tables I and II it will be seen that the composition of the stones does not vary appreciably with the age of the animal that of a calculus from a six-months old calf (stone No 9) or from a 12-year old fully grown-up bullock (stone No 5) being approximately the same The chemical composition also seems to be independent of the place of nativity of the animals as also of their diet So far as these limited observations are concerned, there

seems to be an indication that stones from different provinces do not materially differ either in their chemical composition or in their physical characteristics, notably structure and shape

Calcium and magnesium content of waters drunk by the animals

Samples of water, drunk by five animals suffering from urinary lithiasis, were provided for analysis Calcium and magnesium were determined in them, the results being given in Table IV

TABLE IV

Analysis of the water drunk by the animals

Stone number	CaO Parts per 100,000	MgO Parts per 100,000
1	7 6	2·9
3	3·4	1 6
6	29 9	16 4
7	36·3	39 0
12	2 5	3 3

It is seen from Table IV that the calcium content of the waters varied widely from 2 5 to 36 3 per 100,000 The values for magnesium are equally divergent, these varied from 1 6 to 39 0 parts per 100,000 The chemical composition of the stones seems to have been unaffected by the mineral content of the water drunk by the animals, the composition remains approximately the same whether the stone came from an animal drinking water rich in calcium and magnesium or from one used to a water poor in these two elements

Iron content of cattle stones

The bright metallic sheen in most of the stones suggested that it might be due to the occurrence of iron Quantitative micro-estimation revealed the presence of iron in all the stones in which the test was made This result is shown in Table V

Comparison of the chemical composition of cattle stones with that of human stones

Comparing the cattle stones with human vesical calculi the following are the chief differences —

(1) In human stones the most common constituent *is uric acid* or urate which is completely absent in cattle stones

TABLE V

Stone number	Iron (Fe) in milligrams per cent
1	Not examined
2	Not examined
3	Not examined
4	11 0
5	12 5
6	19 2
7	32 0
8	Less than detectable amounts
9	6 6
10	39 1
11	Less than detectable amounts
12	Not examined
13	Trace
14	Trace
15	15 4
16	Less than detectable amounts
18	30 5
19	Not examined
20	Not examined
21	Not examined
22	Not examined
23	Not examined
24	Not examined

(2) Cattle stones contain, on an average, more calcium and magnesium than human stones

(3) Human stones contain much oxalate and little or no carbonate, while cattle stones contain plenty of carbonate with little or no oxalate

(4) Human stones contain much more phosphates and nitrogen than cattle stones

A comparison of the mean composition of human and cattle stones are given in Table VI

TABLE VI

	Moisture Per cent	Total nitrogen Per cent	P_2O_5 Per cent	CaO Per cent	MgO Per cent	C_2O_3 Per cent	CO_2 Per cent
Human stones	7·2	17·9	6·9	14·9	2·0	14·0	0
Cattle stones	3·1	0·4	0·9	44·0	4·8	0·6	39·1

Summary and conclusions

(1) Twenty-three calculi (21 vesical and urethral and 2 renal) from cattle have been analysed

(2) The stones have a fairly uniform composition, consisting mostly of carbonate of calcium with a little magnesium the latter presumably existing as a carbonate

(3) They contain very little nitrogen and no uric acid

(4) The chemical composition of urinary calculi in cattle seems to be independent of the age of the animal, of the place of its residence and of the mineral content of the water drunk by it

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A NOTE ON *BARTONELLA MURIS* ANÆMIA

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(*From the Nutritional Research Laboratories, I R F A, Coonoor*)

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IT has been shown in these laboratories that *Bartonella muris* anæmia—which occurs in albino rats following removal of the spleen—may arise in a proportion of non-splenectomized rats when they are fed on a deficient diet of which the chief faults are deficiency of fat-soluble vitamins and vitamin C (McCarrison, 1927, Wills, 1930) The faulty food would thus appear to induce functional injury to the spleen thereby lowering the resistance to Bartonella infection, in a manner comparable to, though not so effective as, splenectomy It has lately been shown by Perla and Marmorston-Gottesman (1930) that 'the pulp cells of the spleen are specific in the protective mechanism of the rat to *Bartonella muris* anæmia' Our own observations (McCarrison, 1927, Wills, 1930) would appear to indicate that the functional perfection of this mechanism is largely dependent upon the proper constitution of the food

Perla and Marmorston-Gottesman (1930) have recently succeeded in isolating a strain of *Bartonella muris* from normal, non-splenectomized, adult rats by injecting the blood of the anæmic rabbit into young rats, thus demonstrating that the adult rat is a carrier of the virus of *Bartonella muris* anæmia They found, further, that 'splenectomy in suckling rats is not followed by Bartonella anæmia, since during the suckling period the rat is not a carrier of the virus' We have recently made some observations which appear to conflict with this finding, or to show that the new-born albino rat may behave in a different way to the older suckling with respect to this infection

In October 1929, the senior author removed the spleen in 66 young adult rats* Of these, 65 survived the operation and one died in consequence of it With a few exceptions† all developed *Bartonella muris* anæmia—the blood examination being made by Drs Wills and Mehta Of the 65 animals 41 died and 14 survived Amongst the 14 survivors there were 7 males and 7 females These were placed in separate, straw-filled cages and fed on the stock diet in use in these laboratories (whole-wheat flour chapattis lightly smeared with fresh butter, sprouted gram, diluted whole milk, cabbage, carrots and water *ad libitum*, with a small ration of raw meat occasionally) One hundred and eighteen and one hundred and twenty-seven days respectively after splenectomy, one female and one male had a relapse of *Bartonella muris* anæmia from which they died, presumably, they had acquired an immunity to the infection which had worn off by that time Following this incident the six remaining splenectomized females were mated with healthy non-splenectomized males, their tail blood having been first examined for Bartonella with negative results The subsequent history of each of these splenectomized females is given below together with the results of our examination of the blood (usually heart blood) of their offspring —

No S 68 Splenectomized on 19-10-29 Developed *Bartonella muris* anæmia Recovered Mated on 13-5-30 Gave birth to 4 young on 7-6-30 Developed profound anæmia and died on 9-6-30 Blood from heart and smears from liver, taken after death, showed no *Bartonella muris* Post-mortem appearances were characteristic of *Bartonella muris* anæmia (Wills, 1930) All 4 young were killed and eaten by the mother before her death

No S 63 Splenectomized on 19-10-29 Developed *Bartonella muris* anæmia Recovered Mated on 13-5-30 Gave birth to six young on 7-6-30 Killed and ate 4 of them the same day The heart and tail blood of the remaining two was examined for Bartonella which was present in profusion in both (i e, 24 hours after birth)

Mated to another healthy male on 11-6-30 Gave birth to 4 young on 6-7-30 All 4 were killed on 7-7-30 and the heart blood examined for Bartonella with positive results

Mated to a fresh male on 7-7-30 Died of profound anæmia on 29-7-30 Was not pregnant at the time of death Post-mortem appearances were characteristic of *Bartonella muris* anæmia

No S F Splenectomized on 19-10-29 Developed *Bartonella muris* anæmia Recovered Mated on 13-5-30 Gave birth to one young on 8-6-30 Heart blood of the young rat was found to contain Bartonella 72 hours after birth though in relatively small numbers

Mated again on 11-6-30 Gave birth to a litter of 7 on 7-7-30 All young were removed and killed within 12 hours of their birth The heart blood of all showed Bartonella

Re-mated on 7-7-30 Gave birth to a litter of 3 on 31-7-30 Heart blood of all 3 young was positive for Bartonella

Again mated on 31-7-30, the male not having been removed from the cage during her pregnancy Gave birth to a litter of 6 on 28-8-30 The heart blood of all 6 young contained

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*This was done in connection with the investigations on The Anæmias of Pregnancy which were at that time being conducted by Drs Wills and Mehta in these laboratories (Wills, 1930)

† Some were treated in various ways (with liver extract, spleen and vitamin A concentrate) with the object of preventing the development of the anæmia (Wills, 1930)

Bartonella, the organisms being plentiful 24 hours after birth, less so when the rats were two to three days old, and found only sparingly on the sixth day of life

No C 64 Splenectomized on 19-10-29 Developed *Bartonella muris* anæmia Recovered Mated on 13-5-30 No young were born by the 7-7-30 when the male was changed Gave birth to a litter of 5 on 26-7-30 Heart blood of all 5 contained Bartonella within 12 hours of birth

Re-mated on 26-7-30, the male having been left in her cage Four young were born on 19-8-30 Two died shortly after birth The heart blood of the remaining two was examined on the third day of life Bartonella in *small numbers* were present in both

No C 72 Splenectomized on 19-10-29 Developed *Bartonella muris* anæmia Recovered Mated on 13-7-30 Gave birth to one young on 8-6-30, which was killed 3 days after birth, heart blood contained relatively few Bartonella

Re-mated on 11-6-30 No young born up to 7-7-30, when the male was changed Gave birth to 3 young on 24-7-30 Young removed within 24 *hours of* birth Heart blood of all 3 contained Bartonella in abundance Male kept in cage A third litter of 6 was born on 20-8-30 All were removed within 96 hours of birth Heart blood contained Bartonella in all, plentiful at 24 to 36 hours, diminishing in numbers to 144 hours when disappeared

No W 29 Splenectomized on 19-10-29 Developed *Bartonella muris* anæmia Recovered Mated on 13-5-30 No young born up to 7-7-30, when a fresh male was introduced Gave birth to first litter of 5 on 5-8-30 All removed and heart blood examined within 48 hours of birth Bartonella present in *all*

Three interesting facts emerge from these observations (1) Pregnancy is apt to induce a relapse of *Bartonella muris* anæmia in splenectomized albino rats that have recovered from it following the removal of the spleen (2) The heart blood of the one to four-day-old suckling young of splenectomized females invariably contained *Bartonella muris,* though the mothers themselves may not have exhibited symptoms of anæmia which were recognizable clinically; the organisms were most plentiful 24 to 36 hours after birth, thereafter diminishing in numbers until after the seventh day of life they were no longer found (3) *Bartonella muris* may be found in the young of splenectomized mothers within 12 hours of their birth

In view of the finding of Perla and Mormorston-Gottesman referred to above, that 'during the suckling period the rat is not a carrier of the virus,' the heart and tail blood of 23 one to four-day-old rats—the offspring of well-fed and hygienically caged stock animals—were examined for this organism It was found in 5 but not in the remaining 18 So far then as the stock albino rats of these laboratories are concerned we do sometimes (22 per cent) find *Bartonella muris* in one to four-day-old rats We may add, however, that we have so far failed to find the organism after the fourth day of life, and we have the impression—though not desiring to insist upon it—that the infection, in the very young offspring of well-fed and hygienically caged, non-splenectomized rats, is less heavy than in the young of splenectomized animals

It so happened that a large experiment, designed to ascertain the effect of 'dirt' on well-fed albino rats, was at this time in progress These animals were crowded together in wooden-floored cages, of narrow limits, which were never cleaned One female developed *Bartonella muris* anæmia and died from

it after living under these conditions for two months This was the only case* amongst the 180 animals included in the experiment A number of litters were born in these cages and we examined the heart and tail blood of 23 one to four-day-old animals *Bartonella muris* was present in 10 and absent in 13 It would thus appear that conditions of over-crowding and dirt are favourable to infection by Bartonella even in well-fed animals This is no doubt due, in considerable measure, to the multiplication of the rat-louse—the vector of the virus—which occurs in these circumstances, involving, perhaps, a mass dosage of the virus It is within the bounds of possibility that the enlargement of the spleen, which we (McCarrison and Newcomb, 1930) have noted in rats living in dirty cages, may be a protective reaction against this infection

An interesting observation made in very young rats born of well-fed, non-splenectomized parents living in clean cages was that while one or more members of a litter showed *Bartonella muris* in the blood the others did not This is in striking contrast to the results observed in the new-born young of splenectomized mothers, in these *Bartonella muris* was invariably present in the heart blood of each member of the litter

Summary

1 Rats that have recovered from *Bartonella muris* anæmia, following splenectomy, may develop the disease again at a later date and succumb to it although during the intervening period they have been well-fed, hygienically caged and isolated from contact with other animals Relapse occurred in 14 2 per cent of our spleenless animals

2 Pregnancy is apt to induce another attack of *Bartonella muris* anæmia in rats that had previously suffered, and recovered, from it following splenectomy

3 The one to four-day-old offspring of splenectomized mothers invariably showed *Bartonella muris* in the heart blood The organism was found in the blood as early as 12 hours after birth

4 The blood of suckling rats, born of non-splenectomized mothers and aged between 1 and 4 days, is not invariably free from *Bartonella muris* even though the parents be well-fed and hygienically caged One member of a litter may be infected, another not The organism was not found in these sucklings over four days old

5 Over-crowding and dirt are favourable to *Bartonella muris* infection even in well-fed animals

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*It must here be added that about the time this case occurred the animals were not receiving their usual *abundant* supply of carrots owing to a temporary shortage of the vegetable Amongst rats in colonies there are always some who do not get their fair share of the ration.

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OSTEOMALACIA (LATE RICKETS) STUDIES

Part V

OSTEOMALACIA IN THE KANGRA DISTRICT

BY

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In a previous communication cases of late rickets and osteomalacia have been described among both sexes in India, comparable to the post-war 'Hunger osteomalacia' in Central Europe These cases were seen during a visit to the Kangra valley where the disease is known locally as '*chinji oi*' denoting a joining together of bone Observations on 83 patients showed that not only is the vitamin-content of their diet insufficient, but the calcium, phosphorus and all other constituents are also qualitatively deficient (Wilson, 1929, Wilson and Patel, 1930, Wilson and Surie, 1930*a* and 1930*b*)

The mission hospitals at Palampur and Kangra recorded the frequency of the condition among their patients, who were found at different age periods to show the various signs of early rickets, late rickets and of osteomalacia respectively It was decided to carry out a more detailed survey in this district and the records of this undertaking are embodied in the present paper

The Kangra valley—The district of Kangra which includes besides Kangra proper, Kulu, Lahul and Spiti, with the neighbouring Simla Hill States form the south-eastern part of the Punjab Himalayan tract Kangra proper is one of the most densely populated districts in the Punjab in proportion to its cultivated area, it is more agricultural and more essentially Hindu than any equal tract of country The average elevation of cultivation and habitation is rather less than 3,000 feet For half the year the climate is warm and moist, the rainfall being not less than 100 inches per annum The soil shows a deficiency in lime, phosphorus and magnesia (Lander *et al*, 1929) In many parts cultivation is difficult as the land has to be cleared of stones and needs terracing Domestic animals are generally badly nourished and the inhabitants of poor physique

It appears that not only is the incidence of grazing animals too intense but the density of the human population is also excessive (Report of the Conservator of Forests, Eastern Circle, Punjab, 1930) Of the total 2,544 square miles in Kangra much is uninhabited mountainous country, only 769 square miles are cultivated, so that the pressure on the cultivated area is 837 persons to the square mile It is evident that the population throughout the tract has reached a limit which already exceeds the capacity of the country to support

The marriage of young girls is frequent Statistics show that at the age period 10–14 one in every three girls is married,* while at the age period 5–9 the figures are one in twelve (*Punjab District Gazetteers*, VII, Part A, Kangra District, 1924-25) The new Kangra saying 'Rs 100 for every year of the bride's age' merely exaggerates an undoubted fact that the bride's value increases with her age (Darling, 1930)

Incidence of osteomalacia—It is difficult to state the incidence of rickets in its various forms, early rickets, late rickets and osteomalacia, in a district where villages are scattered, means of communication very difficult and medical relief rarely obtained Dispensary returns show that early cases are not diagnosed, help is only obtained at childbirth in very difficult labour cases and often not then One village when visited reported that 5 osteomalacic women had died in childbirth during the previous six months, fortunately a sixth case was persuaded to come to a mission hospital for Cæsarean section At Palampur Mission Hospital where an outlook is kept for the disease and cod-liver oil freely given, 177 women and children came for treatment from 39 different villages and hamlets, the majority from within a radius of ten miles, but some from an area eighteen miles distant, during the first six months of the year (1930) Cases of osteomalacia have been reported by the mission doctors when on tour in all parts of the Kangra valley as well as in Kulu and in Mandi State Since the Kangra district lies in the Himalayan tract it is to be expected that goitre will be prevalent About a quarter to one-third of the patients attending the Palampur Mission Dispensary show evidences of goitre, but the condition does not appear more frequently among osteomalacic than ordinary patient, nor does improvement in osteomalacia following treatment coincide with any decrease in the goitre Judging from the many cases of osteomalacia studied in other areas in the Punjab where goitre is rarely seen, goitre cannot be considered a factor in the ætiology of osteomalacia

Survey of Launa—The area chosen for individual inquiry was Launa village adjacent to Palampur (N latitude 32 7°, E longitude 76 35°, feet above sea-level 4,300), in the northern part of the district, lying at the foot of the main range of the outer Himalayas, the Dhaula Dar, which here attain an altitude of over 15,000 feet At Launa the lower hills dissolve in the valley in gentle grass-covered slopes

* Personal observation would suggest four in five as more correct

Two crops yearly are obtained, chiefly rice, maize and wheat, flax also is grown in winter There are numerous tea gardens in the district Inquiries show the extraordinarily small amount of vegetables or fruit grown* A reason given for this, in a country where such cultivation would be easy, is the lingering influence of the old Rajput tradition in the Kangra district Rajput warrior clans who were the ruling race had a prejudice against agriculture, and did not utilize vegetables the lower classes have unconsciously followed this tradition in their diet Also certain orthodox Hindus consider such vegetables as onions and carrots unclean As showing the strength of this habit, it is noteworthy that 17 patients from the Kangra district met with at osteomalacia clinics at Lahore and Simla, although living in towns where vegetables were cheap and fruit inexpensive, were found to maintain the same limited dietary as in Kangra

The 38 homesteads which comprise Launa village are scattered over the grassy knolls of the hill-side, some distance behind them are higher hills which form a semi-circle limiting the head of the valley—above this are towery snowy peaks, in front the valley slopes down gently towards Palampur Round Launa are few trees and the whole village is freely exposed to sunlight The primitive houses, composed of mud-brick with stone foundations and wooden rafters, are of double or single storey, animals and their owners live in close association There is usually a small courtyard, and also a small piece of land among the neighbouring fields belonging to the family

As previously recorded, in those cities of the Punjab where osteomalacia is prevalent, a consistent lack of vitamin D, either due to want of sunlight or to an unbalanced diet, was found to be the predominant factor in the ætiology, confirming the experimental findings in other parts of the world Greater severity of the disease among Mohammedan women observing purdah was also noted (Wilson and Patel, 1930) In Launa village all the inhabitants live freely in the open air, being accustomed to sit outside their houses when their hours of work in the open field are completed Sunshine is plentiful for eight months in the year, in the remaining four months are rains and mist, and during this period patients state that their pains are worse The men have usually lived in Launa village all their lives, but their wives may have come from some neighbouring village

The 38 village families fall into three groups, Chumars, Zemindars and Gurkhas The average number per household varies from 1 to 8 The

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* Palampur tahsil, total population, 131,000
Crops in acres

	Summer		Winter
Rice	34,000	Wheat	34,000
Maize	18,000		
Tea	8,500		
Vegetables	200		200

condition on examination was classified according to the degree of severity (+, ++, +++) These headings are comparable to those used in examination of osteomalacia patients in other districts of the Punjab (Wilson and Surie, 1930*a*) The visible deformities noted were epiphyses, bossing, stunted growth, deformity of pelvic or other bones, gait Inquiry was made regarding sites and degrees of severity of pain

I Chumars—The lowest social class in the village, occupation coolies, i e, field labourers Each family has a very little land of their own but the men work chiefly in other people's fields, getting paid by a share in the produce The women and children work in the neighbouring tea fields all the year round The men occasionally may make a little extra income by following their hereditary craft in shoe-leather work Children receive no schooling except at a mission night school The earnings being often in kind are difficult to estimate, one family of six members, and another of four, state they made Rs 12 per mensem by field work

Daily diet—Milk, none or occasionally, or when the cow is not dry (The small ill-nourished animals give very little milk) Eggs, nil Fresh fruit and greens, nil Atta, amounts vary from 8 oz to 1½ lb per head (The wheat is hand-ground) Dal, about 2 oz daily Rice, 8 oz to 2 lb per head Cooked vegetables, once weekly, or sometimes, or occasionally in spring and summer Meat, a very little once a week, or once or twice a month Ghee or oil,* about ⅛ to ¼ ounce daily per head It was noted that children occasionally are in the habit of eating earth, lime or charcoal

Incidence of symptoms—Twenty-four households consist of 111 members, of these 109 members were examined, 40 males and 43 females showed symptoms

II Zemindar class—Chiefly Hindu Khatris, but including zemindars who are respectively Rajput, Mohammedan (converted Hindu), and a Batwal (low caste) Hindu They usually work on their own land but may have some quite distinct, fairly well-paid, trade, such as carpentry Some of the boys have gone to High School

Daily diet—Milk, sometimes Eggs, nil Fresh fruit and greens, nil Atta, 1 lb to 1½ lb per head Dal, 2 oz per head Rice, 1 to 2 lb per head Cooked vegetables, several times a week Meat, once or twice a month Ghee and oil, 2 to 4 oz daily per head

Incidence of symptoms—Ten households, with 51 members, 47 were examined, of these 8 males and 8 females showed symptoms

III Gurkhas—Families have regimental pensions and have bought land

Daily diet—Milk, 2 to 8 oz daily per head Eggs, nil Fresh fruit and greens, occasionally Atta, 4 oz to 1½ lb per head Dal, 1 to 2 oz per head Cooked vegetables, once or twice daily this class can afford to buy

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*The oil is from mustard or flax seed

imported bazaar vegetables Meat, once or twice weekly or oftener Ghee, ¼ to 1 oz daily, extra oil is used in cooking

Incidence of symptoms—Four households with 13 members, 7 males and 6 females, none showed symptoms

TABLE

Rickets and osteomalacia among inhabitants of Launa village Relation of caste, age and sex to severity of clinical symptoms

Degree of severity	Under 17 years = Rickets Clinical symptoms			Over 17 years = Osteomalacia Clinical symptoms			Total
	+++	++	+	+++	++	+	
GROUP I Chumars 109 persons examined							
Male		6	16	1	7	10	40
Female	2	3	12	11	11	4	43
GROUP II Zemindars 47 persons examined							
Male			8				8
Female		1	4		2	1	8
GROUP III Gurkhas 13 persons examined							
Male							
Female							

Treatment—Women and girls with pains and deformity were asked to attend a special centre at the Palampur Mission Hospital daily for a course of treatment of three weeks duration It was not easy for women workers to come regularly and their attendance was irregular Three forms of treatment were started

1 Fruit + vitamin D
2 Fruit + milk 20 oz
3 Fruit + milk 16 oz + vitamin D

Milk, owing to diseases prevalent in the neighbourhood, was given boiled Fruit consisted of two large oranges or their equivalent in some other form,

e g, mango, melon, etc Vitamin D was given in the form of irradiated ergosterol, i e, Ostelin or Vigantol *

Results—Patients on diets 1 and 3 showed improvement, diet 3 appearing the most satisfactory It was thought that cases on diet 2 showed some improvement, but the women themselves were discontented, since their pains did not clear up as quickly as those of their companions on the other diets

A second course of treatment, diet 3 for three weeks, was given to those who could come It was noted that the addition of some form of vitamin C, such as fruit or fresh greens, to the diet of an osteomalacic patient appears clinically to hasten improvement (Wilson and Surie, 1930*b*), while the addition of vitamin A (milk) leads to improvement in the nourishment of the patient Nine patients who attended regularly daily for six weeks, on examination at the end of that period, were found to be entirely or very nearly free from pain Muscular spasms were also much relieved so that deformity was lessened, and patients were enabled to move about more freely †

There was a marked improvement in the general nourishment of the patients, some women formerly thin now looking quite plump It was estimated at current Palampur rates, for a patient to keep herself on the level of diet 3 for a month, the cost of living would be Rs 17 to Rs 18 per head

Conclusions—The Table shows the incidence and severity of symptoms found at the different age periods, early rickets, late rickets and osteomalacia, among Chumars, Zemindars and Gurkhas respectively Every household at Launa village obtained a very fair amount of sunlight, and the symptoms appeared to be due to the fact that the diet, as shown by the analysis previously given, could not contain any appreciable amount of the precursor of vitamin D for the sunlight to act upon

The greatest incidence of the disease occurs among the Chumars whose diet shows quantitatively and qualitatively deficiency, with a possible excess cereal factor in addition Among them the disease occurs throughout all age periods, showing the strain of adult working life on both sexes, with increased severity among the women in connection with childbirth and prolonged lactation Out of 24 households only 2 escaped without symptoms, a study of these is significant, one household consisted of two members only, the other of three members had an additional source of income in that the husband had been taught carpentry by the mission

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* Cod-liver oil was not used in this experiment since some Hindu patients object to animal oil, and in others the digestion tends to be upset Hospital patients, however, who during the same period were treated with cheap (i e, not too refined) cod-liver oil showed quite satisfactory, though slower, improvement

† One husband joyfully announced that his wife, who had been unable to walk about, could now do her household work and carry up the family water supply from a spring some distance away

There is a marked decrease in incidence and in severity of symptoms among the zemindar class Their more favourable economic condition was shown by the addition to their diet of milk sometimes, cooked vegetables several times weekly, and fairly adequate amounts of animal and vegetable fat (ghee and oil)

Gurkhas living under favourable economic conditions showed no symptoms They had an adequate amount of milk, cooked vegetables and fats in their diet Except in the case of the last group who bought imported vegetables, and occasionally fruit, all articles of diet were obtained locally

The results of treatment show the great improvement in symptoms which follow the addition of vitamin D to the diet of those suffering from pain and deformity

Preventive measures—This disease among the poorer classes in the Kangra district is so intimately bound up with economic conditions that apparently it could only be stamped out by far-reaching measures against over-population, such as adult instead of early marriage

Suggestions for improvement—(1) Encouragement of vegetable cultivation round homesteads by the Agricultural Department, Punjab, with provision of suitable seeds, e g, cabbage, cauliflower, spinach, brinjals, turnips, soya bean, etc

(2) Better diagnosis at hospitals and dispensaries so that patients may be encouraged to come earlier for treatment, and for the lowest classes the free provision of some substance containing vitamin D *

(3) Propaganda Women belonging to such classes as can afford to improve their household dietary, should be taught how to do this, their husbands, if literate, can be supplied with suitable pamphlets of instruction (This is already done at the Palampur Mission Hospital with encouraging results)

Probably the most useful propaganda is through the younger generation Many Hindu boys attend school, and now gradually little girls are being sent to school also Miss Balderston of the Canadian Mission has written a very simple but entertaining little drama in the vernacular on the subject of osteomalacia prevention, which has been successfully acted on various occasions by the school girls at Palampur This little drama has been printed in Hindi by the Rural Community Board, Punjab, and through the kindness of the Deputy Directress of Public Instruction, Punjab, has been distributed, to be read in all the girls' schools in the Kangra valley

Summary—1 Rickets in its various stages, early rickets, late rickets, and osteomalacia, is very clearly associated with great pressure on the available means of subsistence in the Kangra district

2 Suggestions are put forward for the prevention of this disease

* Suitable cod-liver oil can be delivered in Kangra at a cost of Rs 7-4 per gallon

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OSTEOMALACIA (LATE RICKETS) STUDIES

Part VI

FACTORS IN TREATMENT

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OBSERVATIONS during the treatment of 267 cases at the osteomalacia clinics at Lahore, Amritsar and Simla showed that the best results were obtained when (*a*) *vitamin D* (some form of irradiated ergosterol such as Ostelin or Vigantol, or a cod-liver oil which contained that vitamin) was combined with (*b*) the daily use of *milk* (not less than 8 ounces), i e, vitamin A, fats and salts, (*c*) *fresh fruit* or greens, i e, vitamin C, (*d*) *sunlight* (sitting or lying out of doors with as much direct exposure as possible for not less than two hours), i e, stimulation of vitamin D production in the skin, and (*e*) *massage* (using some bland oil as lubricant), i e, circulatory stimulation The diet of these patients was shown to be unbalanced, but usually contained an adequate amount of vitamin B, an excess cereal factor might be present (Wilson and Surie, 1930*a*) It was noted that after commencement of treatment there may be a refractory period up to about three weeks, and when clinical improvement occurs it is found to be associated with a change in the blood picture (Wilson and Patel, 1930) During the course of these studies experiments have been made with the use of Indian fish oils and also attempts to get irradiated substances manufactured on a commercial scale in India—but so far unsuccessfully.

The present investigation was undertaken to estimate the value of the various factors in the 'combined treatment' which unfortunately has the disadvantage of being relatively expensive The chief object was, if possible, to determine some form of relief within the means of poorer patients

Methods—During the period of this inquiry 107 women, girls and children of various races, Hindu, Mahommedan and Sikh, suffering from rickets in its different forms, osteomalacia, late rickets and early rickets (Wilson, 1929), attended three special clinics at Lahore Two clinics were held in the city, the third in a separate area containing the homes of industrial workers Observations showed that the diets of these patients were unbalanced and, in addition to their housing conditions, sunlight was often deficient These patients, while continuing to live in their own homes, were divided for purpose of treatment into five groups, and the results were controlled by repeated X-ray examinations

Treatment—Group I *Diet*—The daily addition of vitamins A and C, fats and salts Milk* 16 to 30 ounces, according to the amount the patient could digest, following Indian usage the milk was taken boiled Fruit consisted of two large fresh oranges grown locally

In order to check results certain women regularly partook of this diet under observation

Group II *Sunlight*—Two hours out in the sun daily with direct exposure of at least the affected parts A number of patients, who were offered sun and privacy on the roof of a Welfare Centre, were controlled by daily observation

The difficulty of obtaining facilities for exposure to sunlight by city women and those who observe purdah has been explained (Wilson and Surie, 1930*a*) Observations on the intensity of the ultra-violet solar radiation made by various observers using Hill's acetone-methylene-blue gauge in different Indian towns give reading in 'units' per day varying according to the season from 3 to 12 These readings are for exposure in the open, in the small dark room which is the poorer city-woman's home the results of exposure are nil Many school children owing to over-crowded and unsuitable school buildings also suffer from lack of sunlight (Wilson and Surie, 1930*b*)

Group III *Vitamin D*—This was given for convenience in the form of Ostelin or Vigantol in 9 to 12 daily

Group IV *Diet* + *Sunlight*—These were used under conditions similar to Groups I and II

Group V *Sunlight* + *Vitamin D*—These were used under conditions similar to Groups II and III

To ensure regular clinic attendance by patients who demanded 'medicine,' Pil Blaud one b d was given to each patient in Groups I, II and IV The choice

* This milk was the best available within reach of the patient In the city the cows were kept in or near the vendor's homes at night but sent far out for grazing by day, in the industrial areas pasteurized milk was obtained from a Government dairy

of iron was determined by the anæmia which in some degree is present in most city cases

Familial tendencies—Familial tendencies to osteomalacia, late rickets and early rickets have been previously recorded (Wilson and Suiie, 1930*a*) It is interesting that in connection with these 107 patients similar cases were noted in 39 family groups and households by personal observation at the clinics, the actual incidence was probably much higher One family group of Mohammedans observing purdah who lived in neighbouring city homes, numbered among them 13 cases of rickets in its various forms, including six advanced cases of osteomalacia

Results of treatment—Among the total 107 patients in this investigation 66 attended the clinics for a period less than the three weeks which, as explained previously, owing to a refractory period is the shortest interval for observation of treatment These included all the cases of early rickets as well as those cases of late rickets and osteomalacia who lived at a distance and only visited the clinics for advice The remaining 41 patients, women and girls, attended regularly for at least three weeks, and many were kept under observation for 2 to 5 months, they were suffering from moderate or severe late rickets and osteomalacia corresponding clinically to the classes ++ and +++ previously described (Wilson and Suiie, 1930*a*)

The *X*-ray appearances in osteomalacia have already been described (Wilson, 1929, Pilley, 1929) In the cases under observation, before starting treatment the *X*-ray picture showed bones mottled in appearance with a general loss of detail, the differentiation between cortex and medulla was less clear than normal, and the cortex of the bone was becoming thinned from within outwards Fractures were often present usually sub-periosteal and without much displacement The bones often showed longitudinal striations

Clinical improvement in osteomalacia, evidenced by lessening of pain and muscular spasms, occurs before any change can be seen radiographically, but gradually as the disease is arrested the general outline of the bones on *X*-ray examination becomes more clearly defined, and the cortex is reconstituted, the new bone being usually denser than normal, especially in the region of fractures

Group I *Diet*—Ten patients, all put on weight, 7 of these, who were very poor and living under most unfavourable conditions, had no lessening of pain or spasms, but after the three weeks of observation were completed, very rapidly improved on the addition of vitamin D The remaining three patients belonged socially to the middle class, living under much more favourable surroundings and in the habit of obtaining sunlight, their symptoms improved so much that it was unnecessary later to supplement treatment with vitamin D

Group II *Sunlight*—Five patients, all wives of industrial workers on small wages having a very unbalanced diet, showed no obvious improvement, when after three weeks treatment vitamin D was added, their symptoms cleared up slowly

Group III *Vitamin D*—Twelve cases improved gradually under treatment

Group IV *Diet + Sunlight*—Seven cases improved slowly under treatment

Group V *Sunlight + Vitamin D*—Seven cases improved under treatment considerably more rapidly than Group III

Massage does not effect any cure of itself, but many patients state it gives considerable temporary relief, especially if carried out before lying down at night when the pain is usually most severe

Conclusion—The importance of an unbalanced diet in the ætiology of osteomalacia and late rickets is shown by these results Improvement in diet only leads to improvement in symptoms when sunlight is available (Group I), conversely the provision of sunlight for cases on deficient diets is of little avail (Group II), this fact has also been confirmed by observations on osteomalacia in the Kangra district (Wilson, 1931) The results of Groups III, IV and V show that, in order to get more rapid improvement, it is advisable to combine the use of vitamin D with daily exposure to sunlight and an improved diet There is also much value in training patients in a better mode of life (Group V)

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OSTEOMALACIA (LATE RICKETS) STUDIES

Part VII

RICKETS AMONG INDIAN CHILDREN OF SCHOOL AGE

BY

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Introduction—It is evident that an investigation of the incidence of a disease presupposes the correctness of the diagnosis Recently the clinical conception of rickets has undergone a radical change With the use of *X*-rays for early diagnosis and the estimation of the organic phosphorus of the blood, well-established clinical signs have been re-appraised on the basis of objective criteria, and it has become possible to compare in different countries rickets in its various forms, infantile rickets, late rickets and osteomalacia The geographical distribution of rickets, and the incidence and relative severity of its various forms in different parts of the world is given by Hess (1930) The results of recent investigations in many lands show the importance in ætiology of lack of sunshine, together with social and economic factors (Wilson and Surie, 1930*a*) With the application of specific measures to prevent its development, the area of the distribution of the severer forms of rickets in civilized countries is becoming more and more circumscribed

The percentages given for the proportion of rickets among children of school age in various countries and even in neighbouring parts of the same country vary considerably, obviously apart from different local conditions, much depends on a personal equation in the estimation of the lesser degrees of deformity (Kerr, 1926) There is, however, general agreement that infantile rickets is more frequent in boys than girls, and that even a moderate degree of rickets is often associated with a lack of vitality and resistance to disease which

constitutes one of the most important dangers of this disorder Mentally, rachitic school children are dull, and mostly below standard in their studies (Newsholme, 1922) It is also recognized that there is a kind of physiological rickets among quickly growing school children which if taken in time, is easily compensated by anti-rachitic protective measures (Paton and Findley, 1926) Interesting facts emerge from an examination by Sterling of over 5,000 children between the ages of 6 and 14 years among the negro population, who in the United States are peculiarly liable to rickets At the age six to seven years twice as many boys as girls showed bony evidences of rickets, but at the age of 14 the reverse relationship was found, the signs of rickets began to predominate in girls 10 to 11 years old Also twice as many cases of rickets occurred among girls in the age group of 14 and over, as in girls of the age group 6–7 years Such increase indicates the onset of late rickets among the girls (Sterling, 1928)

Sex incidence and severity of rickets among Indian children—Previous work in connection with this osteomalacia (late rickets) inquiry has shown that, in certain areas where predisposing factors are present in marked degree, the disease may be met with among both sexes and at all ages in both rural and urban areas Such extreme conditions are found among field workers in the Kangra district (Wilson, 1931) Late rickets also occurs among adolescent boys at Srinagar in Kashmir, a city known for the high incidence of osteomalacia among a large section of the female population who, living amidst great poverty, in damp over-crowded houses, are subjected to the rigors of a severe winter (Vaughan, 1926) We noted on viewing 500 school children (300 boys, 200 girls) at Srinagar, that for children under 10 years of age the signs of rickets were more marked among boys, yet at the later age period 10–17 years the disease was much more evident and active among girls, although many boys at this period showed marked enlargement of epiphyses at wrists and ankles and curving of the lower legs Among the girls there was a marked difference in the incidence and severity of rickets found among the pupils of two adjacent high schools in Srinagar city The improvement in the Mission High School was probably associated with the holding of open-air classes, and the provision of milk and cod-liver oil when required, under the direction of the school doctor

An unusual incidence of rickets was met with by us among certain industrial workers or their families In Amritsar, adolescent boys, working in carpet factories under far from satisfactory conditions, showed bossing and marked
-lment of epiphyses at wrists and ankles, combined with flat foot, bent legs,
nees and deformities of tarsal bones associated with their cramped posi-
orking with feet pressed up against the looms In Bombay, a city in
s apparently free from rickets (Hutchison and Patel, 1921), the
r visiting different groups of children was to show the presence of
rked degree among the families of low grade industrial

Apart from such exceptions, usually in India late rickets and osteomalacia exert their effects, deformity and pain, on women and girls in cases where insufficient sunlight is associated with an unbalanced diet, although infantile rickets may be observed among both sexes Among 158 cases under 17 years of age, observed at special clinics in Lahore, Amritsar and Simla, only 18 were boys

During an investigation at Simla (Wilson and Surie, 1930*b*) on the incidence of dental caries among 103 rachitic school girls (44 of whom were under 10 years of age, and 59 between the ages of 10 and 17 years), causative factors of rickets (want of sunlight owing to home customs and to over-crowded unsuitable school buildings, together with an unbalanced diet), were found among girls of every social class The satisfactory development of girl prisoners, attending school in the Lahore women's jail, may be ascribed to their balanced diet, and adequate provision of sunlight

The value of a carefully planned, even if simple and cheap, diet, if combined with plenty of sunlight, was demonstrated among the children of a mission industrial school in a village near Lahore, only 19 out of 185 girls showing slight signs of rickets

Observations in Lahore city schools—In view of the great demand for female education in Punjab cities and the consequent danger of over-crowding, observations were made regarding the incidence of rickets among girls during

TABLE I

Results

Schools	5–10 YEARS		10–17 YEARS	
	Girls examined	Cases of rickets	Girls examined	Cases of rickets
Class I—				
Mohammedan Middle	179	143	48	37
Government High	330	126	226	116
Class II—				
Mohammedan Middle	117	61	57	27
Hindu Middle	177	27	64	20
Class III—				
Mission High	205	32	79	18
TOTAL	1,008	389	474	218

school life in a city, Lahore, where clinically late rickets and osteomalacia are frequently seen

Methods—The schools were divided into three classes

1 Inside the city walls, in very congested areas

2 Extra-mural, in less crowded neighbourhoods

3 Situated in ample grounds

The *ages* of the girls examined varied from 4 to 19 years The majority of girls were under 13, since after the 7th class wastage due to marriage is considerable

The *deformities* chosen as indicative of rickets were bossing, enlarged epiphyses at wrists and ankles, knock-knee, curving of the lower legs, and associated irregularity of dentition Examination was easy because of the interest aroused in both teachers and pupils, since to both the crippling effects of late rickets and osteomalacia were well known

Home conditions—In every class inquiries were made regarding diet, recreation, rest and sleep

TABLE II

Racial distribution of rickets

Class I Government High School	Number of girls	Cases of rickets
Hindu	354	159
Mohammedan (observing purdah)	202	83

Discussion—The highest incidence of rickets is found among the girls attending class I intra-mural city schools, with their homes in adjacent areas The most severe rickets is amongst the lower-middle class Mohammedan girls going to a school held in an ancient adapted building with very over-crowded stuffy rooms, which were also darkened in order to ensure privacy and purdah Here in one class of girls 5–10 years old, out of 55 children 33 had rickets, while the teacher was suffering from osteomalacia There was much rickets among the girls in a popular over-crowded high school, especially in the 10–17 year age period Table II shows no marked difference in the racial distribution of rickets in this school where Hindu girls reading Hindi, and Mohammedan girls reading Urdu, worked side by side It was noted that three Hindu school girls, who were sisters, all had rickets

As already stated (Wilson and Surie, 1930*a*) in a crowded city, Hindus who do not keep purdah may suffer as do purdah-observing Mohammedans, from want of sunlight

But the difference in incidence of rickets among Hindu and Mohammedan girls in the class II extra-mural schools shows that in less crowded neighbourhoods the evil effects of the deprivation of sunlight by purdah customs become more evident The Hindu school was very crowded and the rooms dark, but the classes were able to overflow on an adjacent open space The Mohammedan school building was not crowded, was airy and very clean, but there was very little sunlight owing to the necessity for purdah

Children need material for construction of new tissues, and their diet should be considered from the point of view of adequacy to support optimum growth In both classes of schools more use might have been made of modern knowledge of dietetics in advising children in the need of the regular intake of milk and fresh fruits, which in many cases the parents could have well afforded The need for properly carried out medical inspection of school girls is obvious Whenever there is over-crowding bad attitudes are adopted during school hours, and it is obvious that rachitic children suffer most from ill-fitting school furniture

A low incidence of rickets was found in the class III school, held in a large well-ventilated building with free access to sunlight, situated in extensive grounds, where great care was taken in limiting the number of pupils to prevent over-crowding in classes The day pupils though largely Christian included many well-to-do Hindu and Mohammedan girls, and the small amount of rickets found was fairly equally distributed in the three groups Familial incidence among the pupils was noted in several cases The unfavourable effect of home influence was shown by the occurrence of cases of rickets among a group of boarders, who while at school received an admirably balanced diet, and much sunlight

As regards the effects of higher education, among the total 1,482 school girls examined, 22 girls were working for matriculation, of these 11 girls (6 out of 8 girls in class I, and 5 out of 13 in class III) had rickets On enquiry some of these girls admitted to doing long hours of homework and obtaining but little rest and sleep

Conclusions—1 The sex incidence and severity of rickets among Indian children is discussed, and the existence of late rickets among boys in certain areas and in certain industries, where the predisposing factors are present in a marked degree, is noted

2 In an area (Lahore city) where late rickets and osteomalacia among girls and women bring about deformities especially of the lower extremities and of the bony pelvis which have serious consequences at childbirth, a high incidence of rickets, 607 cases, were found on examination among 1,482 school girls

3 The association of rickets with unfavourable health considerations in schools is shown Suggestions for improvement are put forward, based on what is already being successfully carried out in certain Indian schools

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OSTEOMALACIA (LATE RICKETS) STUDIES

Part VIII

ADULT SPASMOPHILIA

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BIOCHEMISTRY has amplified the views of pathologists as to the pathogenesis of infantile rickets, late rickets and osteomalacia, and their identity Studies of blood chemistry in children with rickets show that the essential abnormality is a low inorganic phosphorous content The serum calcium is usually normal but in a small proportion of cases it is reduced, and in such cases tetany (spasmophilia) may occur (Hunter, 1930) The blood chemistry of osteomalacia has been studied in China by Miles and Feng (1925) In their cases osteomalacia was found to resemble 'low calcium' rickets, and the frequency of tetany was noted, there was a considerable diminution of the serum calcium to 5 0 to 7 4 mg per 100 c c, the diffusible calcium varied in its behaviour The plasma phosphorus varied from 1 8 to 3 8 mg per 100 c c

It may happen that a low serum calcium is encountered in cases which do not show any active or latent tetany The explanation offered for this apparent anomaly is that any process which tends to produce an acidosis increases the ionization of calcium, so that although the calcium value may be low, the ionized fraction is within normal limits (Davies, 1930) Among those suffering from the post-war hunger osteomalacia in Central Europe were many elderly patients of either sex who complained of cramp in feet, legs or forearms, and tetany was not infrequent, adolescents of the age period 14–20 years also suffered (Dalyell and Chick, 1921)

Hess (1930) has collected facts regarding the world distribution of rickets, osteomalacia and tetany Various observers during the past fifty years state that these conditions are especially prevalent in Vienna, and that cases there are more severe than in the large cities of Germany, it is suggested that the wretched

housing conditions in Vienna are probably important factors in causation As regards the sex incidence of tetany, it is noted that tetany in adults, as in infants, occurs far more often in males than females, and has a seasonal incidence in winter and spring

Adult spasmophilia in India—The occurrence of tetany among women and girls suffering from osteomalacia and late rickets in India has been recorded by various observers (Scott, 1916, Stapleton, 1915, and Hutchison and Stapleton, 1924) In the present communication adult tetany in connection with these conditions among men and boys is described, and the relationship is considered of the idiopathic tetany or *wai* of Kashmir, and the endemic tetany found in goitrous districts of the Himalayas mentioned by Castellani (1909), to the various manifestations of rickets

Methods—The incidence of tetany among cases of late rickets and osteomalacia met with in connection with this osteomalacia (late rickets) inquiry has been noted, together with associated symptoms of pain and bony deformity The patients were under observation for various periods up to two years, their ages varied from 10 to 55 years

Tetany during the course of late rickets and osteomalacia may occur at any age, whenever some extra strain is placed on the organism Though often associated with pregnancy or lactation, in many cases some other debilitating factor such as an intercurrent illness, enteric fever, etc, is stated to have preceded the onset of the spasmophilia In osteomalacia and late rickets tetany ceases, apart from treatment, with subsidence of the acute condition, but exacerbations in the course of chronic cases of considerable duration occur repeatedly, on the addition of some unfavourable condition, e g, a subsequent pregnancy The association of muscular spasm with deformity in late rickets and osteomalacia has been mentioned (Wilson, 1929) Irritability of the tendons on the dorsum of the foot is frequently seen in late, as in infantile, rickets It was observed during the examination for rickets of Indian children of school age (Wilson, 1931*b*), and was often noted among 50 rachitic school girls over 10 years of age, examined at Simla during an inquiry on the incidence of dental caries (Wilson and Sutie, 1930*b*), though typical tetany was present in only one girl aged 10 years Infantile tetany is rare in India, a possible explanation being the prolonged lactation and breast feeding given by Indian mothers The children of osteomalacia mothers rarely show any clinical sign of rickets until near the end of the second year

When tetany is present, the spasms vary much in frequency—occurring daily, once or twice a week, or occasionally when startled, or with some strain such as attempting to carry a heavy weight Spasms are always worse in cold or damp rainy weather The tetany may be either in hands or feet alone, or carpopedal spasm may be present Slighter degrees of spasmophilia, cramps of varying severity, numbness or tingling, may occur in hands or feet alone or associated with tetany in another part, and Trousseau's sign is easily obtained Where carpopedal spasm is present, Chvostek's sign may be elicited

and the hyper-irritability of the facial nerve may be such that twitching of the entire side of the face or merely the muscles of the alæ nasi and mouth or of the eye on the corresponding side, is complained of by the patient

In the histories of onset obtained from patients, occasionally tetany was stated to be the first symptom noted This is of special interest in view of certain cases kept under observation at the osteomalacia clinics, where at the first visit tetany was the only sign found on examination, though at subsequent visits a year or more later, slight tilting of the pelvis was detected and the bone pains of osteomalacia were present There is no doubt that in areas where late rickets and osteomalacia occur, such cases in their early stages are labelled hysteria, and appropriate treatment withheld

Results—Cases of tetany recorded during the present inquiry fall into three classes

(1) Cases seen at osteomalacia clinics at Lahore, Amritsar and Simla
(2) Cases among field workers in the Kangra district
(3) Cases of *wai*, the so-called idiopathic tetany of Kashmir

TABLE

	Cases of late rickets and osteomalacia in patients over 10 years of age		Tetany associated with bone pains and deformity		Tetany associated with deformity		Tetany	
	Male	Female	Male	Female	Male	Female	Male	Female
Class I—								
Lahore, Amritsar and Simla		397		191		1		1
Class II—								
Kangra district	39	96	3	47	2	2		1

The relative importance of sunlight and inadequate diet as ætiological factors have already been stated (Wilson and Surie, 1930*a*), also the reasons why goitre cannot be considered a factor in the ætiology of osteomalacia in the Kangra district explained (Wilson, 1931*a*)

Certain observations on the blood chemistry of some Lahore patients in class I have been published (Hughes, Shrivastava and Sahai, 1929) Estimations of the serum calcium and inorganic phosphorus were made on 11 untreated cases of osteomalacia and on 18 cases who had been under treatment for varying periods Normal values for serum calcium were found in mild or early untreated cases, and also in treated cases which had attended the clinics for a considerable period Certain acute cases showed low values for both

serum calcium and inorganic phosphorus A rise in the serum calcium might precede clinical improvement, but the latter was associated with a rise in the inorganic phosphorus Low calcium values were associated with tetany At Bombay, in a series of 20 cases of osteomalacia in pregnant women, the clinical condition was shown to be closely related to the inorganic phosphorus level of the blood (Private communication, *b*)

Class III—Through the kindness of certain doctors, cases of tetany called *wai* in Kashmir were selected for examination Some of the stigmata of past rickets, bossing and enlarged epiphyses, or the presence of slight osteomalacia with a small amount of bone pain and tilting of the pelvis, was noted in every case observed It has been shown previously that associated with the frequency of osteomalacia in Kashmir is a high incidence of rickets (of moderate severity, florid rickets in children is uncommon) at all age periods (Wilson, 1931*b*) *Wai* is found frequently in pregnant or lactating women, but may occur among young girls, and in connection with menstruation, it is often preceded by diarrhœa Tetany may be very severe, in addition to carpopedal spasm, spasms of the feet and intercostal muscles are frequent, while all parts of the body may be affected The incidence is probably higher during cold weather, though fewer cases come out to hospital for treatment during the severe winter months The racial incidence varies in different areas, *wai* occurs among Hindus as well as among purdah-observing Mahommedan women, among the well-to-do, as well as amongst the very poor It is said very occasionally to be met with among men As noted by Vaughan regarding osteomalacia (Vaughan, 1926), *wai* is rarely met into among the *mangis,* Mohammedan boat women who are much in the open air and have a plentiful supply of greens and vegetables Among *wai* patients the diets of both well-to-do and poor were deficient, the poor could not afford milk and but rarely obtained fruit, greens or vegetables, for cooking they used vegetable oils. It was not the custom among the better class of patients to use milk, they rarely ate fruit or vegetables, but had a relatively large amount of meat in their diet Both classes of patients used much hand-husked rice, but the latter class also added some flour to their diet and used animal fat in the form of ghee for cooking The unfavourable housing conditions in Kashmir have already been described (Wilson, 1931*b*) As regards the condition of teeth, among the population generally there is much pyorrhœa, and the teeth tend to drop out

Goitre is common in Kashmir, but no special incidence has been observed among *wai* cases The total hardness of Srinagar drinking water is 16 parts per 100,000 (Private communication, *a*)

Treatment—Among hospital cases favourable results are obtained from the use of cod-liver oil, altered diet, and tonics The success following the use of magnesium sulphate, in doses of 2 drams daily for 2 or 3 weeks is of interest in view of the intestinal intoxication theories in the causation of tetany (Hess, 1930) It is interesting to find that some *hakims* advise a fish diet and milk and certain fruits in the treatment of *wai*

Discussion—It is obvious, since tetany associated with late rickets and osteomalacia has an age, sex and seasonal incidence, that the number of cases of tetany seen must be influenced by these factors In the above results three forms of tetany are noted (*see* Table)

(1) Associated with bone pains and deformity, (2) with deformity alone or (3) tetany without any characteristic signs of late rickets or osteomalacia In class I few cases are seen under groups 2 or 3, since patients, originally coming under these groups, later developed the characteristic symptoms of group I

Among class II patients in the Kangra district, tetany among both sexes is found associated with late rickets and osteomalacia, but the incidence of tetany is much higher among females The relationship of certain cases of *wai* to rickets and osteomalacia is evident from the results of class III

In all three classes the importance of balanced diet and adequate sunlight becomes evident, while the results of classes II and III show that goitre in the Himalayas cannot be considered an ætiological factor

Conclusions—1 The incidence of tetany among women and girls suffering from osteomalacia and late rickets in different areas of the Punjab is considered It is noted that when tetany is a prominent symptom, early cases tend to be diagnosed and treated as hysteria

2 Adult spasmophilia is described in connection with cases of late rickets and osteomalacia, among boys and men in the Kangra district

3 The ætiology of certain cases with tetany called *wai* in Kashmir, and their relation to late rickets and osteomalacia, is discussed

4 It is suggested that these conditions may be similar to those described by Castellani as endemic tetany of the Himalayas

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OSTEOMALACIA (LATE RICKETS) STUDIES

Part IX

DISTRIBUTION

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PREVIOUS investigators have recorded cases of late rickets and osteomalacia in certain towns of northern India and Kashmir (*see* References quoted in Part III of the paper, *Ind Jour Med Res*, XVII, 3, p 901), recent methods permitting the diagnosis of early and of less severe cases have extended the knowledge of this disease (Pilley, 1929, Wilson, 1929) Ætiological factors determine the distribution of rickets in its various forms, osteomalacia, late rickets and early rickets Investigation of the diet, housing and customs of such cases during the course of the present inquiry has shown that a consistent lack of vitamin D, either in connection with diet alone, or due to lack of sunlight, was a predominant factor in ætiology, confirming the experimental findings in other parts of the world (Wilson and Patel, 1930) Climate is a secondary factor, but whenever the disease is present, the seasonal incidence of damp and cold augments the condition Investigation of factors in treatment indicates the importance of food values, since a rapid improvement on the addition of vitamin D is only obtained if vitamins A and C are included in the diet, while excess cereal appears to exert an adverse influence (Wilson and Surie, 1930*a*) Certain observations on the blood chemistry of these patients show the varying content of serum calcium and inorganic phosphorus at different stages of the disease (Hughes, Shrivastava and Sahai, 1929) The blood picture suggests a deficiency condition, while the examination of stools and urine indicate the possibility of an infective process in the intestine either as a primary factor, or dependent on an avitaminotic condition (Wilson and Surie, 1930*b*), the study of adult spasmophilia in connection with late rickets and osteomalacia indicates that intestinal conditions may cause exacerbations (Wilson, 1931*a*)

Distribution of cases—The results of the present inquiry fall into two groups, rural and urban

1 *Rural*—Occasional cases of rickets and of osteomalacia associated with pregnancy were met with personally among village women of the northern

India plains or noted by certain doctors and health visitors in country districts A high incidence of rickets and osteomalacia at all ages in both sexes among Hindu field workers was found to be closely associated with pressure on the available means of subsistence in the hill country of the Kangra district (Wilson and Coombes, 1931) Doctors have reported osteomalacia among Pathan women of the nomadic *powindah* tribes of the North-West Frontier Province and among Mohammedan women in distant Kashmir villages (Private communication, *a*)

2 *Urban*—At special clinics in Lahore, Amritsar and Simla, 400 cases of late rickets and osteomalacia and 61 cases of infantile rickets were kept under observation and examined In addition records of 103 hospital cases of osteomalacia were obtained through the kindness of doctors at Amritsar, Bhiwani, Delhi, Ferozepore, Gujranwala, Lahore and Simla The incidence of rickets was high in over-crowded and walled cities and in towns with narrow lanes and high sunless houses, as in Delhi and Gujranwala, or in densely populated bazaars such as Simla, among Hindu and among purdah-observing Mohammedan girls and women In Ludhiana a town with a very low incidence, where careful inquiry over two years by doctors and health visitors only revealed five indigenous cases of rickets and osteomalacia (Private communication, *b*), it was found that the diet of the average household did not differ materially from that of women in a city such as Amritsar where the incidence for all forms of rickets is high, but in Ludhiana over-crowding was absent, the brick and mud-brick houses stood separate, and plentiful sunlight was available even for purdah women, in open courtyards

As the result of the examination of over 2,000 children of school age, rickets in towns was found to be associated with lack of sunlight either in over-crowded schools, and with diet also in certain industrial areas (Wilson, 1931*b*) Dental caries, a disease ætiologically closely allied to rickets, was found to be more widespread among Indian children than previously supposed (Wilson and Suire, 1930*b*)

Discussion—The racial distribution shows that rickets and osteomalacia are found in northern India and Kashmir in towns wherever there is deficient sunlight combined with an unbalanced dietary, and in rural areas whenever plentiful sunlight cannot compensate for a diet quantitatively and qualitatively deficient There is no caste or race distinction, a large number of urban cases occur among women and girls in fairly good social circumstances, and a familial incidence is often present The disease may start at any age (54 years was the highest age of onset in the present series), but the sex incidence shows that in late rickets and osteomalacia the chief strain falls on girls and women during adolescence and maternity, except in conditions of extreme deficiency as in the Kangra district, or in certain industries where men and boys are also affected Infantile rickets (under 5 years of age) occurs fairly equally among boys and girls It is rare for the children of osteomalacia mothers to show signs of rickets until the second year The onset may be at any age (*see* Table),

TABLE

Factors influencing the onset in 400 cases of late rickets and osteomalacia from clinics at Lahore, Amritsar and Simla

Race and sex	Male	Female	Age of onset: Before union of epiphyses = late rickets — Years	Age of onset: Before union of epiphyses = late rickets — Cases	Age of onset: Over 17 years, after epiphyseal union = osteomalacia	Relation of onset to marriage: Before	Relation of onset to marriage: After	Associated with pregnancy, lactation or abortion	Associated with illness	Deficient — Sunlight (housing and purdah)	Deficient — Diet	Social condition of family: Poor	Social condition of family: Fair	Social condition of family: Good
Hindu (Brahmin, Rajput, Kayasth, Jain, Khatri, Bunia, Arora, Chumar and Mater)	5	204	5–10	33					Fever, 4 Pneumonia, 3 Cholera, 2 Intestinal infection, 2 Smallpox, 2 Disability after accident, 2 Measles, 1 Abdominal T.B., 1	A familial incidence was observed in 100 cases		Coolies, menial workers	Shop-keepers, mechanics, clerks	Merchants, lawyers, officials
Mohammedan [Sayed, Punjabi, Hindustani (U P), Kashmiri]	1	150	10	18										
Sikh		29	11	14										
			12	25						240	160	209	174	17
Indian Christian		11	13	29										
	6	394	14	34										
			15	32										
			16	41	174	148	252	218						
				226										

but many cases start in connection with reproduction (pregnancy, lactation and abortion), occasionally the disease is noted as having started after some illness Menstruation is usually normal unless some other condition such as anæmia is also present The age at marriage as given by patients with late rickets and osteomalacia may be very low (from 4 years upwards), but actually the patient rarely lives with her husband till after the onset of puberty at about the age of 13 years

Conclusions—1 In northern India and Kashmir rickets in its various forms, osteomalacia, late rickets and infantile rickets tends to occur in any race or caste wherever there is extreme deficiency of sunlight or diet, or more frequently where there is a relative deficiency in both these factors

2 Conditions influencing the onset such as sex, age, marriage, illness, and social conditions are considered

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Dr R Munro, Ludhiana	Private communication (*b*)

A NEW SPECIES OF HUMAN MICROFILARIA (*MICROFILARIA ACTONI* SP NOV) FROM EASTERN INDIA ALLIED TO MICROFILARIA OF *ACANTHOCHEILONEMA PERSTANS*

BY

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DURING the course of the examination of blood from convicts of jails in Bengal to ascertain the incidence of filarial infection, the author came across a solitary slide with many microfilariæ which differed very distinctly from those of *Filaria* (*Wuchereria*) *bancrofti* which is the only species of human filaria so far known from India These microfilariæ were very small in size, and devoid of a sheath The specimen of blood was obtained from a convict, Ali Ahmad, in the Comilla Jail, East Bengal, who came from the village Musapur in the Sandwip Island, at the mouth of the Brahmaputra The author naturally attempted to obtain more slides from this person but, in the interval, this convict was discharged from the jail, having served out his sentence, and all the attempts to get at him since have been futile as the man is suspicious of being taken into custody

Two expeditions were subsequently sent to Noakhali and the Sandwip Island to obtain blood films from this area The result of these surveys was that only the microfilariæ of *Filaria bancrofti* were obtained, and none of this species

The slide from the convict Ali Ahmad is the only specimen available of this species and, ordinarily, one is not justified in describing a new species from a single slide preparation But as the features of these microfilariæ are so very distinct from those of other known human microfilariæ, there seems to be sufficient evidence to show that this is a new species

In the slide at hand, 20 microfilariæ are present and they are all similar in size and in other details The slide was stained first with (1) Giemsa

staining solution and later with (2) Delafield's Hæmatoxylin and Eosin and the details of structure of these microfilariæ have been studied in detail

The following is a detailed description of this new human microfilaria from Eastern Bengal (*see* Plate LII) —

The microfilariæ are devoid of any sheath They have a rounded head-end and tail-end which tapers to a fine point The embryos are 140 μ to 150 μ long averaging about 145 μ The maximum breadth of the body, at about the nerve ring, is 6 μ The body tapers slightly towards the anterior end and the breadth at the tip is 3 μ The nuclei commence some short distance behind the anterior end of the worm, first in two rows, and more posteriorly there is dense aggregation of nuclei up to the nerve-ring There is a sharp break at the nerve-ring, and then posterior to it for a short length, the nuclei are densely packed After about one-third to half the length of the microfilaria, the nuclei are less dense and irregularly distributed The distance from the anterior end to the nerve-ring is 30 μ or one-fifth of the length of the embryo The tail-end of the microfilaria is very characteristic The tail tapers to a fine point, the nuclei occurring to nearly the extreme tip of the tail These nuclei of the tail are long and rod-like and there results a chain of three (sometimes two or four) rod-like nuclei extending to the tip of the tail Another feature of this microfilaria is the absence of the central viscus (Innenkorper)

The author proposes the name *Microfilaria actoni* sp nov for this species, in honour of Lieut-Col H W Acton, I M S , who has contributed largely to the understanding of the pathology of filariasis in India

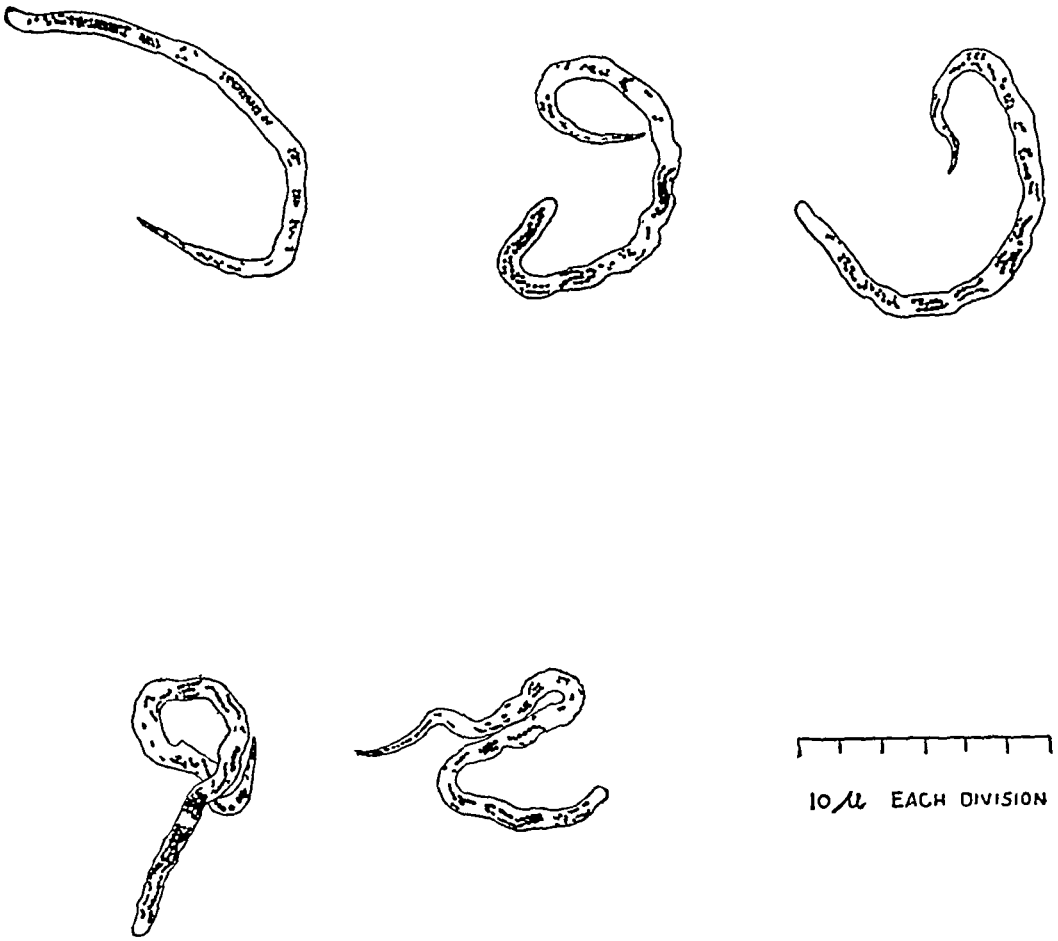
The main characteristic features of *Microfilaria actoni* sp nov are as follows —

(1) Absence of a sheath,
(2) Small size (140 μ to 150 μ long and 6 μ broad),
(3) Absence of a central viscus,
(4) The tail end which tapers to a fine point and has a few long rod-like nuclei extending to nearly the tip of the tail

It would not be out of place here to compare this microfilaria with the other known microfilariæ from man, namely, microfilariæ of *Loa loa, Filaria (Wuchereria) bancrofti, Microfilaria malayi,* microfilariæ of *Acanthocheilonema perstans, F demarquayi* and *Onchocercus volvulus* The first three species have sheathed microfilariæ which measure about 300 μ in length The last mentioned three species, namely *perstans, demarquayi* and *volvulus,* have sheathless microfilariæ These latter species may be compared with the new species described in the present paper

From the above table it is seen that *Microfilaria actoni* is allied to *Microfilaria perstans* The former is readily distinguished from *A perstans* by the much smaller size of the microfilaria and the finely tapering tail-end The latter species has not been recorded from anywhere in the East, being confined entirely to Tropical Africa and British Guiana Oh (1929) has

PLATE LII



Microfilaria actoni sp nov
Camera lucida drawings of microfilaria stained with hæmatoxylin and eosin

recently recorded *F perstans* from Korea, from the finding of sheathless microfilariæ from four cases His reasons for determining these microfilariæ as *perstans* is based chiefly on their small size and the absence of the sheath It is not impossible that Oh was actually dealing with *Microfilaria actoni*

	Microfilaria perstans *	*Microfilaria demarquayi* *	*Microfilaria of O volvulus* †	*Microfilaria actoni* sp nov
Length	197 μ	255 μ	300 μ	145 μ
Breadth	4 5 μ to 5 μ	5 μ	8 μ	6 μ
Position of the nerve-ring	22 per cent			20 per cent
Characters of the tail	Tip rounded stumpy, nuclei present to tip of tail	Tail tapering, tip devoid of nuclei	Sharply pointed recurved tail	Tail finely tapering and acute, nuclei present to tip of tail
Tail nuclei	Round	Round	Round	Rod-like

* The measurements of the microfilariæ of *F perstans* and *F demarquayi* mentioned in this table are based on the work of Vogel (1928)

† The measurements of *O volvulus* microfilariæ are taken from Dyce-Sharp (1926)

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INCIDENCE OF BRUCELLA AGGLUTININS IN AN AVERAGE UNSELECTED INDIAN POPULATION

BY

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AND

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EVER since the Mediterranean Fever Commission in Malta (1904–1907) traced the cause of prolonged, irregular pyrexias amongst troops to the consumption of milk of the infected goats, the medical profession all over the world have begun to realize the importance of the Brucella group of organisms in prolonged fevers of uncertain origin The observations of this Commission were confirmed from time to time and now Malta fever is definitely attributed to *Micrococcus melitensis* Further members of this group have been discovered which are closely related to *Micrococcus melitensis*

B abortus (Bang) was at first regarded as infectious to cattle only, bringing about abortion in those animals, but the work of Keefer (1924), Evans (1924, 1927) and others has definitely established that this organism is also capable of producing prolonged and irregular fever in man Cases are frequently reported where there is strong evidence to show that the human infection was contracted from animals suffering from the disease That in a country like India, Brucella infection may account for a considerable amount of illness is brought home to one when the economical and industrial conditions prevailing here are considered Agriculture being so far the main industry of this country, a vast majority of the population live here under rural conditions The presence of so many hills and pasture lands also afford a good opportunity for breeding cattle and goats both for commercial purposes and for the supply of milk for consumption Even in the present day of scientific treatment of milk before consumption, i e, pasteurization and sterilization by other methods, such milk is only available for an infinitesimal number of the

population, the majority using raw milk Most of the tropical diseases prevalent in India have received a fair amount of consideration from the people and the State but the importance of Brucella infection is not fully realized yet Unfortunately the clinical symptoms of this infection are so varied and misleading that a clinician can hardly regard any one syndrome as typical of these infections The pyrexia alone has been found to be undulant, remittent and intermittent, other manifestations such as sleeplessness, disinclination for work, etc , are equally indefinite

Moreover, primary cultures of the organisms from the patient's blood or urine are rather difficult to obtain It has been stated that even cases showing agglutinins to a titre of 1 in 100 in their blood prove negative by blood culture On the other hand in early cases the patient's serum may not agglutinate the organisms in such a high dilution as to make a definite diagnosis possible by this method

If every blood serum sent up to bacteriological laboratories in this country were examined for Brucella agglutinins, irrespective of the clinician's request, we think a great step will be taken towards the detection of cases who are sources of danger to the community and in a few years time it would be possible to estimate the incidence of Brucella infection

We collected our sera both from our staff and the local population and in addition examined all the sera sent up for routine Wassermann and Widal reactions In all 482 sera were examined 247 being from healthy adults and 135 from febrile cases The Wassermann sera have been included in the healthy series as they were few and not supposed to be from febrile cases

Technique

The agglutination technique of Harvey (1920) was employed with the difference that the Widal racks were incubated for 4 hours at 37°C and the results read over-night Both the agglutinins of *Micrococcus melitensis* and *B abortus* (Bang) were tested for Results are tabulated below —

TOTAL SERA 482

Total healthy cases 247			Total febrile cases 135		
	M melitensis	*B abortus*		*M melitensis*	*B abortus*
Positive 1 in 10 to	11	12	Positive 1 in 10 to	15	12
Positive 1 in 20 to	16	12	Positive 1 in 20 to	6	9
Positive 1 in 50 to and over	6	20	Positive 1 in 50 to and over	8	17
Negative 1 in 10 to	214	203	Negative 1 in 10 to	106	97

The above table shows the presence of Brucella agglutinins in the blood of healthy as well as febrile cases in certain percentages There may be many explanations of the presence of such agglutinins In the healthy individuals the possibility of the positive cases being carriers cannot be overlooked and the febrile cases may be true specific infections

It is not yet commonly agreed at what dilution of serum complete agglutination of this organism indicates Brucella infection Some consider 1 in 50 and 1 in 100 as specific reactions but the fact remains that some such possible carriers are sources of danger to others and should be properly segregated till free of infection Of course the agglutination in lower dilutions may be non-specific or on the other hand may be due to a previous infection or a recent consumption of milk of infected animals

SUMMARY

(1) The sera of 487 cases (healthy and febrile) were tested for Brucella agglutinins by Widal reaction

(2) Both groups of cases in certain percentages showed the presence of Brucella agglutinins

We are indebted to Major G C Maitra, I M S, for his kind advice and criticism of this paper

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SOME ASPECTS OF THE BEHAVIOUR OF CINCHONA ALKALOIDS TOWARDS LIVING CELLS

Part II

PENETRATION OF THE VARIOUS CINCHONA ALKALOIDS INTO COLLOIDAL GELS

BY

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In a previous paper (Kehar, 1930) the effect of the presence of various electrolytes on the penetration of quinine salts was studied on the chemical analogues of living cells It was shown that the penetration of quinine salts through colloidal systems and their permeability into the living protoplasm of *Chara* cells was considerably influenced, depending upon whether the media used were acid or alkaline The stronger acids and alkalis caused more penetration than the weaker ones The degree of penetration was found to be closely related to the chemical nature and systematic position of the electrolytes in the homologous series It was interesting to see that the behaviour of quinine salts on the colloidal systems as well as on the living cells is practically the same in the presence of any particular electrolyte

In view of the possibilities of such a study helping to elucidate the intricate problems of the absorption and permeation of quinine salts in the human body, it was considered advisable to extend this work further and to study the behaviour of the other important cinchona alkaloids which have been used as curatives and prophylactics in the treatment of malaria

I Technique

The technique followed in this investigation is the same as has already been described in a previous paper (Kehar, 1930) except that instead of using test-tubes of an equal bore, glass tubing of the same diameter has been

employed in all these experiments The glass tubing was cut into eight inches long pieces and corked tightly at one end

(*a*) The cinchona alkaloids used in this study were —

(*i*) Cinchonine chloride,
(*ii*) cinchonidine sulphate,
(*iii*) quinidine (base),
(*iv*) quinoidine,
(*v*) quinine (base)

The above were Howard's pure preparations

(*b*) The influence of the following electrolytes has been studied on the permeability of the above alkaloids Sulphuric acid, nitric acid, hydrochloric acid, formic acid, oxalic acid, acetic acid, propionic acid, malonic acid, sodium hydroxide, succinic acid, sodium carbonate, citric acid, sodium nitrate, sodium bicarbonate and barium nitrate

II Results of experiments

The results of these experiments have been summarized in the following tables (Tables I—V) In these tables A and G indicate agar-agar and gelatin respectively

III Discussion of results

(*a*) *The effect of mineral acids, alkalis and salts on the penetration of the alkaloid salts*

Columns 1 to 3 of Tables I to V demonstrate that all the three mineral acids accelerate the penetration of the salts in the following order $HCl > HNO_3 > H_2SO_4$, thus showing that the stronger acids help the penetration of the diffusing alkaloid more than the weaker ones

Columns 4 to 6 of these tables indicate that the alkalis, i e, sodium carbonate, sodium bicarbonate and sodium hydroxide increase the penetration to a still greater extent than the acids, the order found being $NaOH > Na_2CO_3 > NaHCO_3$ The same interesting relationship as observed in the case of acids exist here also, namely, the stronger the alkali the greater the facility it provides to the penetrating alkaloid

It is shown by columns 7 to 9 that whereas the presence of acids and alkalis influences the penetration of the alkaloids to a considerable extent, that of the neutral salts, e g, sodium nitrate, potassium nitrate and barium nitrate does not seem to effect their diffusion in the gel media

(*b*) *Effect of organic acids*

Columns 10 to 12 indicate that in the case of monobasic acids penetration was only increased by the lowest homologue, i e, formic acid, while acetic acid has no effect, and propionic acid, the highest of the three used, hinders the diffusion to some extent This was found to hold good with almost all the alkaloids used

TABLE I

Penetration of cinchonine chloride (in cm)

Electrolytes used	1 NaOH		2 Na_2CO_3		3 $NaHCO_3$		4 HCl		5 HNO_3		6 H_2SO_4		7 $NaNO_2$		8 KNO_3		9 $Ba(NO_3)_2$	
Gels	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
Time in hours 6	0·9	1·1	0·7	1·0	0·6	0·8	0·9	1·1	0·8	1·0	0·6	0·9	0·6	0·8	0·6	0·8	0·6	0·8
„ „ „ 9	1·2	1·4	1·1	1·4	0·9	1·1	1·2	1·4	1·1	1·3	0·9	1·1	0·8	1·1	0·8	1·1	0·8	1·0
„ „ „ 15	1·5	2·0	1·3	1·9	1·3	1·5	1·5	2·0	1·4	1·7	1·2	1·5	1·0	1·4	1·0	1·4	1·0	1·3
„ „ „ 24	1·9	2·6	1·7	2·4	1·6	2·0	1·8	2·5	1·7	2·2	1·5	1·8	1·2	1·8	1·2	1·8	1·2	1·7
„ „ „ 39	2·3	3·0	2·2	2·9	2·1	2·5	2·2	2·9	2·1	2·8	1·9	2·3	1·4	2·2	1·4	2·2	1·4	2·1
„ „ „ 60	2·5	3·4	2·4	3·2	2·3	2·8	2·4	3·2	2·3	3·0	2·1	2·6	1·6	2·5	1·6	2·5	1·6	2·4

Electrolytes used	10 Formic acid		11 Acetic acid		12 Propionic acid		13 Oxalic acid		14 Malonic acid		15 Succinic acid		16 Citric acid		Control	
Gels	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
Time in hours 6	0·7	0·9	0·6	0·8	0·5	0·7	0·8	1·0	0·7	0·9	0·6	0·8	0·9	1·1	0·6	0·8
„ „ „ 9	0·9	1·1	0·8	1·1	0·6	0·8	1·1	1·3	0·9	1·1	0·8	1·0	1·3	1·5	0·8	1·1
„ „ „ 15	1·1	1·4	1·0	1·3	0·8	1·0	1·4	1·7	1·1	1·5	1·0	1·4	1·6	2·0	1·0	1·4
„ „ „ 24	1·4	1·8	1·2	1·7	1·0	1·3	1·7	2·1	1·4	2·0	1·3	1·9	1·9	2·5	1·2	1·8
„ „ „ 39	1·7	2·3	1·3	2·0	1·2	1·6	2·0	2·5	1·7	2·4	1·5	2·3	2·2	2·9	1·4	2·2
„ „ „ 60	2·0	2·7	1·5	2·4	1·4	2·0	2·2	2·8	1·9	2·7	1·8	2·6	2·5	3·2	1·6	2·5

TABLE II

Penetration of cinchonidine sulphate (in cm)

Electrolytes used	1		2		3		4		5		6		7		8		9	
	NaOH		Na_2CO_3		$NaHCO_3$		HCl		HNO_2		H_2SO_4		$NaNO_2$		KNO_3		$Ba(NO_3)_2$	
Gels	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
Time in hours 6	0·7	0·9	0·6	0·8	0·6	0·7	0·7	1·0	0·7	0·9	0·6	0·8	0·5	0·7	0·5	0·7	0·5	0·7
„ „ „ 9	1·0	1·3	0·9	1·1	0·8	1·0	1·0	1·2	1·0	1·1	0·9	1·0	0·7	0·9	0·7	0·9	0·9	0·9
„ „ „ 15	1·3	1·7	1·2	1·6	1·1	1·4	1·3	1·6	1·2	1·4	1·2	1·3	0·9	1·1	0·9	1·2	0·9	1·1
„ „ „ 24	1·7	2·1	1·5	2·1	1·4	1·8	1·6	2·0	1·5	1·9	1·4	1·8	1·1	1·3	1·1	1·5	1·1	1·3
„ „ „ 39	2·0	2·5	1·9	2·5	1·8	2·3	1·9	2·4	1·8	2·3	1·7	2·1	1·3	1·7	1·3	1·8	1·1	1·5
„ „ „ 60	2·2	2·8	2·1	3·0	2·0	2·6	2·1	2·7	2·0	2·5	1·9	2·3	1·5	2·0	1·5	2·1	1·5	2·0

Electrolytes used	10		11		12		13		14		15		16		Control	
	Formic acid		Acetic acid		Propionic acid		Oxalic acid		Malonic acid		Succinic acid		Citric acid			
Gels	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
Time in hours 6	0·6	0·8	0·5	0·7	0·4	0·6	0·7	0·9	0·6	0·8	0·5	0·7	0·7	0·9	0·5	0·7
„ „ „ 9	0·8	1·0	0·7	0·9	0·6	0·8	0·9	1·1	0·8	1·0	0·7	0·9	1·0	1·2	0·7	0·9
„ „ „ 15	1·0	1·2	0·9	1·1	0·8	1·0	1·1	1·3	1·0	1·2	1·0	1·2	1·2	1·5	0·9	1·2
„ „ „ 24	1·2	1·5	1·1	1·3	1·0	1·2	1·4	1·7	1·2	1·5	1·2	1·4	1·5	1·8	1·1	1·4
„ „ „ 39	1·5	1·9	1·3	1·7	1·2	1·5	1·7	2·0	1·5	1·8	1·4	1·8	1·8	2·1	1·3	1·7
„ „ „ 60	1·8	2·4	1·5	2·0	1·4	1·8	1·9	2·4	1·7	2·2	1·6	2·1	2·0	2·5	1·5	2·0

TABLE III

Penetration of quinidine (base) (in cm)

Electrolytes used	1 NaOH		2 Na_2CO_3		3 $NaHCO_3$		4 HCl		5 HNO_3		6 H_2SO_4		7 $NaNO_2$		8 KNO_2		9 $Ba(NO_3)_2$	
Gels	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
Time in hours 6	0·6	0·8	0·5	0·7	0·5	0·6	0·6	0·8	0·6	0·8	0·5	0·7	0·4	0·6	0·4	0·6	0·4	0·6
„ „ „ 9	0·8	1·1	0·7	0·9	0·7	0·8	0·8	1·0	0·9	1·0	0·7	0·9	0·6	0·8	0·6	0·8	0·6	0·8
„ „ „ 15	1·2	1·5	1·1	1·3	1·0	1·2	1·1	1·4	1·1	1·3	1·0	1·2	0·8	1·1	0·8	1·1	0·8	1·1
„ „ „ 24	1·5	1·9	1·3	1·7	1·3	1·6	1·4	1·8	1·3	1·7	1·2	1·6	1·0	1·3	1·1	1·3	1·0	1·3
„ „ „ 39	1·9	2·3	1·8	2·1	1·7	2·0	1·8	2·2	1·7	2·1	1·6	2·0	1·2	1·6	1·3	1·6	1·2	1·6
„ „ „ 60	2·1	2·6	2·0	2·5	1·9	2·4	2·0	2·5	1·9	2·4	1·8	2·2	1·4	1·8	1·5	1·9	1·4	1·8

Electrolytes used	10 Formic acid		11 Acetic acid		12 Propionic acid		13 Oxalic acid		14 Malonic acid		15 Succinic acid		16 Citric acid		Control	
Gels	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
Time in hours 6	0·5	0·7	0·4	0·6	0·3	0·5	0·6	0·8	0·5	0·7	0·4	0·6	0·6	0·8	0·4	0·6
„ „ „ 9	0·7	0·9	0·7	0·9	0·5	0·7	0·8	1·0	0·7	0·9	0·6	0·8	0·9	1·1	0·6	0·8
„ „ „ 15	0·9	1·1	0·9	1·1	0·7	1·0	1·0	1·2	0·9	1·1	0·8	1·1	1·1	1·4	0·8	1·1
„ „ „ 24	1·2	1·5	1·1	1·3	0·9	1·2	1·3	1·6	1·2	1·5	1·1	1·4	1·4	1·7	1·0	1·3
„ „ „ 39	1·4	1·8	1·3	1·6	1·1	1·5	1·6	1·9	1·5	1·8	1·4	1·7	1·7	2·0	1·2	1·6
„ „ „ 60	1·7	2·1	1·5	1·8	1·3	1·7	1·8	2·3	1·7	2·2	1·6	2·0	1·9	2·3	1·4	1·8

TABLE IV

Penetration of quinoidine (in cm)

Electrolytes used	1		2		3		4		5		6		7		8		9	
	NaOH		Na_2CO_3		$NaHCO_3$		HCl		HNO_3		H_2SO_4		$NaNO_3$		KNO_3		$Ba(NO_3)_2$	
Gels	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
Time in hours 6	0·5	0·7	0·5	0·6	0·5	0·6	0·5	0·7	0·5	0·7	0·5	0·6	0·3	0·5	0·3	0·5	0·3	0·5
" " " 9	0·7	1·0	0·7	0·8	0·7	0·8	0·7	0·9	0·7	0·9	0·7	0·8	0·5	0·7	0·5	0·7	0·5	0·7
" " " 15	1·0	1·3	1·0	1·2	0·9	1·1	1·0	1·2	0·9	1·1	0·9	1·0	0·7	0·9	0·7	0·9	0·7	0·9
" " " 24	1·2	1·7	1·2	1·6	1·1	1·5	1·3	1·6	1·2	1·5	1·1	1·4	0·9	1·1	0·9	1·1	0·9	1·1
" " " 39	1·7	2·0	1·6	1·9	1·5	1·8	1·7	2·1	1·6	2·0	1·5	1·8	1·1	1·3	1·1	1·3	1·1	1·3
" " " 60	1·9	2·3	1·8	2·2	1·7	2·1	1·9	2·2	1·8	2·1	1·7	2·0	1·3	1·6	1·3	1·7	1·3	1·6

Electrolytes used	10		11		12		13		14		15		16		Control	
	Formic acid		Acetic acid		Propionic acid		Oxalic acid		Malonic acid		Succinic acid		Citric acid			
Gels	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
Time in hours 6	0·5	0·6	0·4	0·5	0·3	0·4	0·5	0·7	0·4	0·6	0·3	0·5	0·5	0·7	0·3	0·5
" " " 9	0·6	0·8	0·6	0·7	0·4	0·6	0·7	0·9	0·6	0·8	0·5	0·7	0·8	1·0	0·5	0·7
" " " 15	0·8	1·0	0·8	0·9	0·6	0·8	0·9	1·1	0·8	1·0	0·7	0·9	1·0	1·3	0·7	0·9
" " " 24	1·0	1·3	1·0	1·1	0·8	1·1	1·2	1·5	1·1	1·3	1·0	1·2	1·2	1·6	0·9	1·1
" " " 39	1·3	1·6	1·1	1·3	1·0	1·2	1·5	1·8	1·4	1·7	1·3	1·6	1·5	1·9	1·1	1·3
" " " 60	1·5	1·8	1·3	1·6	1·2	1·5	1·7	2·1	1·6	2·0	1·5	1·8	1·8	2·2	1·3	1·6

TABLE V

Penetration of quinine (base) (in cm)

Electrolytes used	1 NaOH		2 Na_2CO_3		3 $NaHCO_3$		4 HCl		5 HNO_3		6 H_2SO_4		7 $NaNO_3$		8 KNO_3		9 $Ba(NO_3)_2$	
Gels	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
Time in hours 6	0·8	1·0	0·6	0·9	0·6	0·8	0·8	1·0	0·7	0·9	0·5	0·8	0·5	0·7	0·5	0·7	0·5	0·7
„ „ „ 9	1·1	1·3	1·0	1·2	0·9	1·0	1·0	1·3	1·0	1·3	0·8	1·0	0·7	1·0	0·7	1·0	0·7	1·0
„ „ „ 15	1·4	1·8	1·2	1·8	1·1	1·5	1·4	1·8	1·3	1·6	1·0	1·4	0·9	1·3	0·9	1·3	0·9	1·3
„ „ „ 24	1·8	2·4	1·6	2·3	1·5	1·9	1·7	2·3	1·6	2·1	1·3	1·7	1·1	1·6	1·1	1·6	1·1	1·6
„ „ „ 39	2·2	2·8	2·0	2·7	2·0	2·4	2·0	2·8	2·0	2·7	1·6	2·1	1·3	2·0	1·3	2·0	1·3	2·0
„ „ „ 60	2·4	3·2	2·3	3·1	2·2	2·7	2·2	3·1	2·1	2·9	1·9	2·5	1·5	2·2	1·5	2·2	1·5	2·2

Electrolytes used	10 Formic acid		11 Acetic acid		12 Propionic acid		13 Oxalic acid		14 Malonic acid		15 Succinic acid		16 Citric acid		Control	
Gels	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
Time in hours 6	0·6	0·8	0·5	0·7	0·4	0·6	0·7	1·0	0·6	0·8	0·5	0·7	0·8	1·0	0·5	0·7
„ „ „ 9	0·9	1·1	0·7	1·0	0·6	0·9	1·0	1·3	0·8	1·1	0·7	1·0	1·2	1·4	0·7	1·0
„ „ „ 15	1·1	1·3	0·9	1·3	0·8	1·1	1·3	1·6	1·0	1·4	1·0	1·4	1·5	1·8	0·9	1·3
„ „ „ 24	1·3	1·7	1·1	1·6	1·0	1·3	1·6	2·0	1·3	1·8	1·2	1·8	1·8	2·4	1·1	1·6
„ „ „ 39	1·6	2·2	1·3	2·0	1·2	1·5	1·9	2·3	1·6	2·3	1·4	2·2	2·1	2·8	1·3	2·0
„ „ „ 60	1·9	2·5	1·5	2·2	1·4	1·8	2·1	2·6	1·8	2·5	1·7	2·5	2·4	3·0	1·5	2·2

Columns 13 to 15 show that all the three dibasic acids used accelerate the diffusion of the alkaloid in the colloidal systems

It appears as if both in the mono- and di-basic series, the range of penetration is somehow related to the molecular weights of the respective homologous series, since the lower the molecular weight* of the homologue, the greater is the extent of penetration and vice versa

The polybasic acid, i e , citric acid, allows the greatest amount of penetration as compared to the mono- and di-basic series

A comparison of the alkaloids with each other shows that, according to their power of diffusion into gel bodies, they can be arranged as follows —

(*i*) Quinine bihydrochloride,
(*ii*) cinchonine chloride,
(*iii*) quinine (base),
(*iv*) cinchonidine sulphate
(*v*) quinidine (base),
(*vi*) quinoidine

This behaviour can be represented in a graphic form, and the following curves show the diffusion of the alkaloids as affected by sodium hydroxide (Text-figure 1) and hydrochloric acid (Text-figure 2) The curves with the other electrolytes are of the same type and can be plotted from the figures given in Tables I—V The curves A, B, C, D and E represent the penetration of cinchonine chloride, quinine (base), cinchonidine sulphate quinidine and quinoidine respectively

The experimental results can be expressed by the equation

$$P^{2} = aT$$

where P is the penetration, T the time in hours and a the latus rectum of the graph

It is evident from the above analysis that the conclusions arrived at by the study of the penetration of these alkaloids into the colloidal media are, with slight variations, practically of the same nature as those observed in the case of quinine bihydrochloride and quinine bisulphate It is interesting to see that in these experiments also the penetration in gelatin gels is greater than in agar gels

It was considered necessary to determine conclusively that the diffusion bands seen in the gels were really due to the alkaloid salts and not to Cl' or SO_4'' ions only To settle this point, the corks of the tubes were removed after

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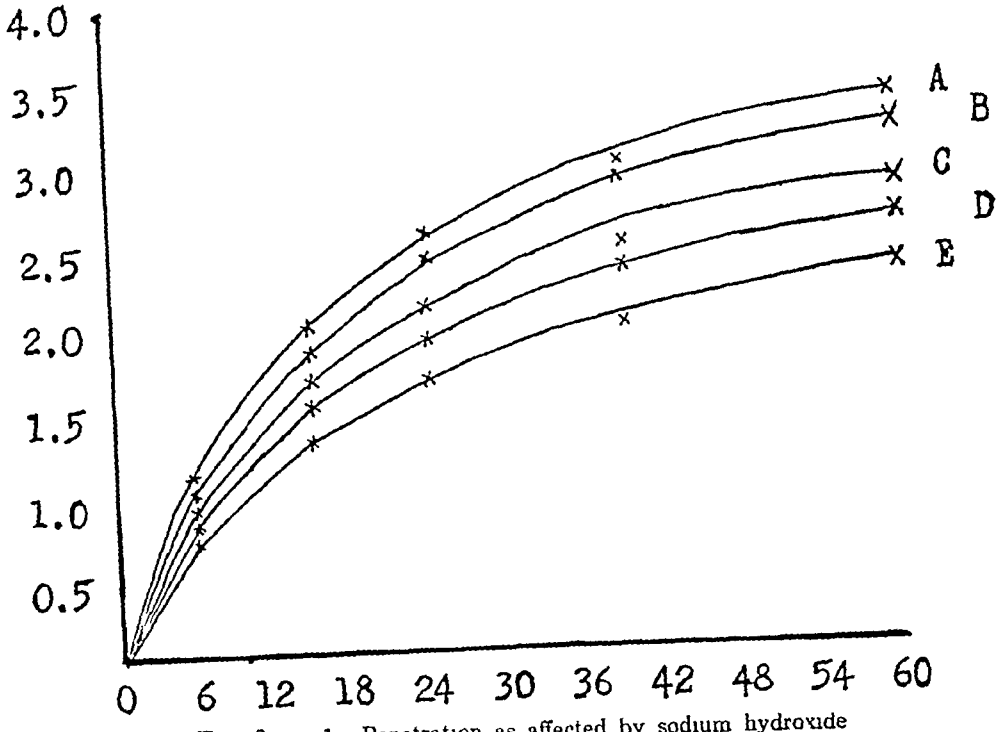
* The molecular weights of the monobasic fatty acids —

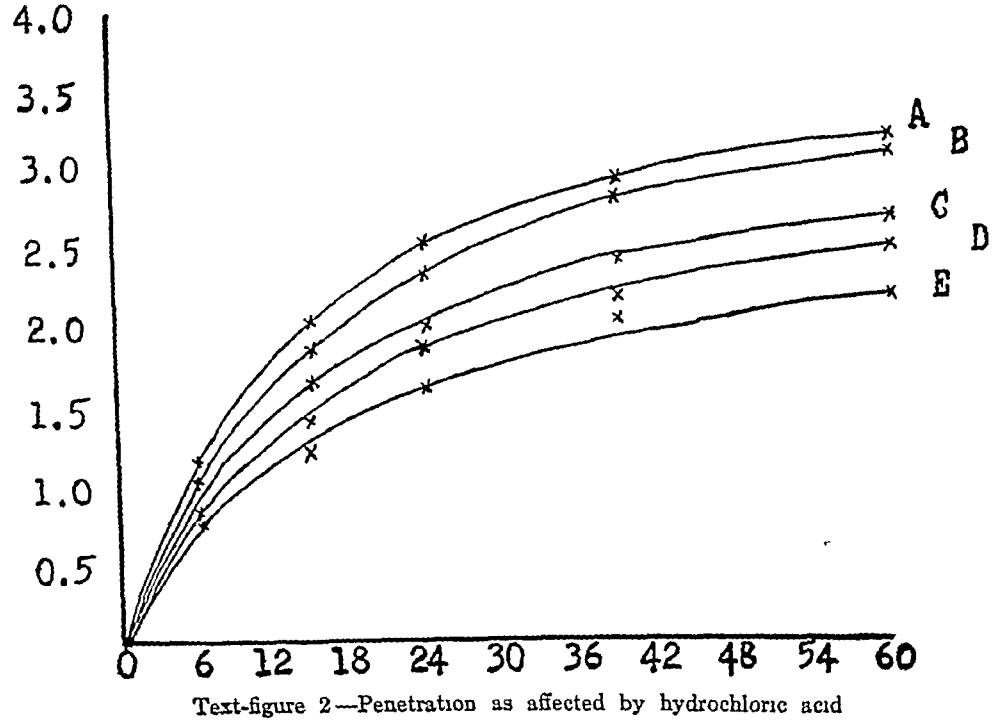
Formic acid	46·03
Acetic acid	60·04
Propionic acid	74·07

The molecular weights of the saturated dibasic acids are —

Oxalic acid (anhyd)	90·06
Malonic acid	104·05
Succinic acid	118·07



Text-figure 1—Penetration as affected by sodium hydroxide



Text-figure 2—Penetration as affected by hydrochloric acid

the experiment was finished and the solid column of gel was removed either by means of a gentle jerk or by passing the tube quickly through hot water Thin transverse slices of the gel were cut with a sharp knife at two or three different places in the area of diffusion These slices were dissolved in distilled water and subjected to the standard tests for cinchona alkaloids It was found that these alkaloids were, invariably, present throughout the body of the diffusion band, except in the case of sodium hydroxide where the alkaloid was sometimes precipitated

This observation was further supported by determining the refractive indices of the alkaloid salt solution above the gel at different intervals during the experiment These readings were taken with the Abbe's Refractometer at 20 07°C, i e, the temperature at which the penetration experiments were being conducted No shift was noticed during the observation period as seen below —

Time in hours	Refractive Index
0	1 3357
6	1 3357
12	1 3357
24	1 3357
48	1 3358
60	1 3357

Control experiments with various concentrations of the alkaloid gave the following results —

Alkaloid used	Concentration Per cent	Refractive Index
Cinchonine chloride	2 5	1 3302
———	5	1 3357
———	10	1 3431
———	15	1 3507
Water alone		1 3122

This shows that, if the alkaloidal solution was not penetrating into the gel as a whole, there would have been a significant change in the refractive indices with changes in concentration

The determination of electrical conductivity of the diffusing salt also gave results similar to the above The observations recorded were the same throughout the experiment, and the readings on the meter did not show any noticeable change

Time in hours	Readings on the meter
0	61 31
6	61 31
12	61 31
24	61 31
48	61 29
60	61 31

Control experiments on the effect of the concentration of the alkaloid on the electrical conductivity were also tried and it was found that the latter changed with variations in concentration

Alkaloid used	Concentration Per cent	Electrical conductivity
Cinchonine chloride	2 5	$0\ 101 \times 10^{-3}$
———	5	$0\ 194 \times 10^{-3}$
———	10	$0\ 374 \times 10^{-3}$
———	15	$0\ 514 \times 10^{-3}$

The foregoing experimental observations give a convincing proof that the alkaloid solutions diffuse through the gel as whole

The structure of the gelatin gel has been a subject of great interest for the colloidal chemists during the early developmental period of this branch of chemistry

Hardy (1899, 1900) states, that these jellies possess a fine open spongy structure, containing fluid within its meshes, resembling more or less a honeycomb structure

A possible explanation of the differences observed in the penetration of the alkaloid may be found in the supposition that the state of aggregation of the tiny capillaries, through which diffusion takes place, is affected differently in the presence of different electrolytes The nature of ions in combination with gelatin and agar gels also has a considerable influence on the forces of cohesion of the solid jelly Loeb (1922) believes that the presence of an acid or alkali raises the osmotic pressure, viscosity and swelling power of a gelatin jelly rapidly, while the addition of neutral salts exerts practically no influence Similar views have also been expressed by Plank (1890) with regard to the influence of osmotic pressure

The hydrogen-ion concentration of the body fluids and tissues is about $0\ 37 \times 10^{7}$, which is conceivably within the range of neutrality The stock gels used for all these experiments were of pH 4 7, i e, the iso-electric point It can, therefore, be anticipated that the behaviour of acids, alkalis and salts observed in the gel media might well have a bearing on the important phenomena occurring in the body under similar conditions

It is now generally accepted that the $H+$ and $OH-$ ion concentration of normal blood and tissues is very nearly close to neutrality, and that all the normal life phenomena occur at a pH about 7, which may be considered equivalent to the iso-electric point of gelatin The reactions taking place therein are accelerated or retarded by the addition of larger amounts of certain organic and inorganic constituents, which cannot easily be neutralized by the body proteins It suggests that under abnormal conditions the pH is altered which is thus mainly responsible for these enhanced biochemical reactions This seems to coincide with the experimental observations The addition of acids and alkalis influences the pH of the gel to a considerable extent and is hence responsible for the greater and quicker diffusion of the alkaloids, while

the neutral salts generally, with few exceptions, do not change the pH of the medium and, therefore, probably exert no appreciable effect It is clear, therefore, that by bringing about a shift in the pH of the body fluids, the velocity of reaction of the drug might be considerably increased

In previous experiments (Kehar, 1930) on the permeability of the alkaloid salts into the protoplasm of the living cells of *Chara*, it was shown that these cells died of the toxic effect of the mineral acids and sodium hydroxide The shift brought about by these reagents in the pH of the cell sap, probably, was incompatible with the life of the cytoplasm So that, whereas the diffusion of the alkaloid could with ease be studied on the chemical analogues of living cells, it was not possible to conduct such a wide range of experiments on living protoplasm

The foregoing results would place the cinchona alkaloids as regards their permeability into the colloidal systems in the following order quinine bihydro chloride, cinchonine chloride, cinchonidine sulphate, quinidine (base) and quinoidine MacGilchrist (1915) tested the effect of the alkaloids in doses proportionate to body-weight in malaria cases and classified them in the following order as regards their clinical effects cinchonine, quinine, quinidine, cinchonidine, and quinoidine, the first three being nearly equal in their action Fletcher (1925) from his experiments places them in the order, quinine, quinidine, cinchonine, cinchonidine and quinoidine as regards their effect on the parasites in the peripheral blood He thinks the first three about equal in value, but that cinchonidine is slightly inferior The results recorded here would place quinidine in the fourth position, if permeability is any index of clinical value, which would agree with the findings of Sinton (1930) on the relative effects of these drugs in the production of a permanent cure

The penetration of the alkaloids as shown above is enhanced in the presence of certain electrolytes This might be taken as a possible factor in the reported value of the quinine alkali treatment of malaria advocated by Sinton (1923) The increased permeability would possibly be associated with a more rapid and complete absorption of the drug as suggested by Acton and Chopra (1925)

Summary

The penetration of cinchonine chloride, cinchonidine sulphate, quinidine, quinine (base) and quinoidine into the chemical analogues of living cells has been studied in the presence of various electrolytes It was observed that —

(*i*) The acids and alkalis accelerate the penetration of the alkaloid to a considerable extent

(*ii*) The stronger acids and alkalis allow more penetration than the weaker ones

(*iii*) The neutral salts are generally without any appreciable effect

(*iv*) In the mono- and di-basic acid series the range of penetration of the alkaloids is related to their chemical nature and systematic position in the homologous series

(*v*) The degree of penetration of these alkaloids seems to be approximately an index of their clinical value

ACKNOWLEDGMENTS

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SOME ASPECTS OF THE BEHAVIOUR OF CINCHONA ALKALOIDS TOWARDS LIVING CELLS

Part III

THE EFFECT OF PLASMOQUINE ON THE PENETRATION OF THE CINCHONA ALKALOIDS

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In 1926 a new drug, plasmoquine, was introduced in the treatment of malaria Since that time numerous experiments carried out with this substance have shown that it forms a marked advance in malarial treatment The work with this drug has been summarized by Sinton and Bird (1928) and by Sinton, Smith and Pottinger (1930) The present conclusions with respect to this drug are (*a*) that it has a marked destructive action on the gametocytes of *P falciparum* but not on the schizonts, thus being in marked contrast to the cinchona alkaloids, (*b*) that like quinine it has a marked action on all forms of *P vivax* and also probably of *P malariæ*, (*c*) that it is more effective than the cinchona alkaloids in the production of a permanent cure in chronic benign tertian malaria and (*d*) that better results in the production of permanent cure are obtained in the latter disease by the combination of plasmoquine with the cinchona alkaloids than by either separately

In view of these findings it seemed advisable to investigate the action of this drug on the rates of penetration of the different cinchona alkaloids into gel media

I Technique

The technique followed here has been fully described before (Kehar, 1930, 1931) and all experiments were conducted under similar conditions

The alkaloids tried in combination with plasmoquine were —

(*i*) Quinine bihydrochloride,
(*ii*) quinine (base),
(*iii*) cinchonine chloride,
(*iv*) cinchonidine sulphate,
(*v*) quinidine (base)

One per cent solution of plasmoquine was mixed in equal proportions with the alkaloid salt solutions (5 per cent) prepared as described before The resultant solution was 0 5 per cent of plasmoquine and 2 5 per cent of the cinchona alkaloid under test

II Results of experiments

The results of these experiments have, for brevity, been summarized in Tables I—VI In these tables A and G indicate agar-agar and gelatin respectively

III Discussion of results

From the above tables it is obvious that the general behaviour of the various electrolytes in affecting diffusion through the gel media in the presence of plasmoquine alone or of plasmoquine in the presence of cinchona alkaloids, is practically of the same type as that obtained with the various cinchona alkaloids only (Kehar, 1930) It will be noticed that the mineral acids and alkalis accelerate the penetration of the diffusing liquid, while the neutral salts are generally without any effect The influence of the presence of mono-, di-, and poly-basic acids, based on their chemical nature in the respective homologous series, had a characteristic effect on diffusion

It is of interest to note that whereas the various salts of cinchona alkaloids, when tried alone, can be arranged in a particular order [quinine bihydrochloride > cinchonine chloride > quinine (base) > cinchonidine sulphate > quinidine (base) > quinoidine] according to the extent of their diffusion, the order changes somewhat when they are in combination with plasmoquine The cinchonine chloride combination penetrates more than the one containing quinine, the order with the different alkaloids being —

(*i*) Plasmoquine and cinchonine chloride,
(*ii*) plasmoquine and quinine bihydrochloride,

TABLE I

Penetration of plasmoquine alone (in cm.)

Electrolytes used	1 NaOH		2 Na_2CO_3		3 $NaHCO_3$		4 HCl		5 HNO_3		6 H_2SO_4		7 $NaNO_2$		8 KNO_3		9 $Ba(NO_3)_2$	
Gels	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
Time in hours 6	0·6	0·8	0·5	0·7	0·5	0·6	0·7	0·9	0·6	0·8	0·5	0·7	0·4	0·5	0·4	0·5	0·4	0·5
" " " 9	0·8	1·0	0·7	0·9	0·6	0·8	0·9	1·1	0·7	1·0	0·7	0·9	0·6	0·7	0·6	0·7	0·6	0·7
" " " 15	1·1	1·5	1·0	1·2	0·9	1·1	1·2	1·6	1·0	1·3	1·0	1·2	0·8	0·9	0·8	0·9	0·8	1·0
" " " 24	1·4	2·0	1·3	1·7	1·2	1·5	1·5	2·1	1·3	1·6	1·2	1·5	1·0	1·3	1·0	1·3	1·0	1·3
" " " 39	1·8	2·5	1·7	2·2	1·5	2·0	1·9	2·6	1·6	2·1	1·5	2·0	1·2	1·5	1·2	1·5	1·2	1·5
" " " 60	2·0	2·7	1·9	2·5	1·7	2·3	2·1	2·8	1·8	2·4	1·7	2·2	1·4	1·8	1·4	1·8	1·4	1·8

Electrolytes used	10 Formic acid		11 Acetic acid		12 Propionic acid		13 Oxalic acid		14 Malonic acid		15 Succinic acid		16 Citric acid		Control	
Gels	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
Time in hours 6	0·6	0·8	0·5	0·6	0·3	0·4	0·6	0·7	0·5	0·6	0·5	0·6	0·7	0·9	0·4	0·5
" " " 9	0·7	0·9	0·7	0·8	0·5	0·6	0·7	0·8	0·6	0·7	0·6	0·7	0·9	1·1	0·6	0·7
" " " 15	0·9	1·1	0·9	1·0	0·7	0·8	1·0	1·2	0·9	1·1	0·8	1·0	1·1	1·5	0·8	0·9
" " " 24	1·2	1·4	1·1	1·3	0·9	1·1	1·3	1·6	1·2	1·5	1·1	1·4	1·4	2·0	1·0	1·3
" " " 39	1·5	1·8	1·3	1·5	1·1	1·4	1·6	2·0	1·5	1·9	1·3	1·8	1·8	2·5	1·2	1·5
" " " 60	1·7	2·1	1·5	1·8	1·3	1·7	1·8	2·2	1·7	2·1	1·6	2·0	2·0	2·8	1·4	1·8

TABLE II

Penetration of plasmoquine and cinchonine chloride (in cm)

Electrolytes used	1		2		3		4		5		6		7		8		9	
	NaOH		Na_2CO_3		$NaHCO_3$		HCl		HNO_3		H_2SO_4		$NaNO_3$		KNO_3		$Ba(NO_3)_2$	
Gels	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
Time in hours 6	16	18	13	16	12	14	15	17	13	15	12	14	10	12	10	12	10	12
" " " 9	19	22	17	20	16	19	1·8	2·1	16	19	15	18	12	15	12	1·5	12	15
" " " 15	23	28	21	25	20	24	22	27	21	21	19	23	15	19	15	19	15	19
" " " 24	27	33	26	30	25	29	26	32	25	29	21	28	17	23	17	21	17	23
" " " 39	32	37	30	36	29	34	32	37	28	3·5	28	33	20	26	20	27	20	26
" " " 60	36	43	34	41	32	39	3·7	4·3	33	40	3·1	38	21	3·1	21	3·2	21	31

Electrolytes used	10		11		12		13		14		15		16		Control	
	Formic acid		Acetic acid		Propionic acid		Oxalic acid		Malonic acid		Succinic acid		Citric acid			
Gels	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
Time in hours 6	12	14	10	12	09	11	13	16	12	15	11	11	1·5	17	10	12
" " " 9	14	17	12	15	11	14	1·6	20	15	18	11	17	18	21	12	15
" " " 15	18	21	15	19	14	18	22	25	20	24	18	23	22	27	1·5	1·9
" " " 24	21	25	17	23	16	21	26	30	25	29	23	27	27	32	17	23
" " " 39	25	29	21	27	19	25	29	36	28	31	27	31	33	37	20	26
" " " 60	29	34	25	3·2	22	29	34	41	32	40	30	38	36	12	21	31

TABLE III

Penetration of plasmoquine and quinine bihydrochloride (in cm)

Electrolytes used	1 NaOH		2 Na_2CO_3		3 $NaHCO_3$		4 HCl		5 HNO_3		6 H_2SO_4		7 $NaNO_3$		8 KNO_3		9 $Ba(NO_3)_2$	
Gels	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
Time in hours 6	1·5	1·8	1·1	1·5	1·0	1·4	1·4	1·6	1·2	1·5	1·1	1·4	0·9	1·1	0·9	1·1	0·9	1·1
„ „ „ 9	1·8	2·1	1·5	1·9	1·4	1·8	1·7	2·0	1·5	1·8	1·4	1·7	1·1	1·4	1·1	1·5	1·1	1·4
„ „ „ 15	2·2	2·7	2·0	2·4	1·8	2·3	2·1	2·6	2·0	2·3	1·9	2·1	1·4	1·8	1·4	1·8	1·4	1·8
„ „ „ 24	2·6	3·2	2·4	2·9	2·3	2·8	2·5	3·1	2·4	2·8	2·3	2·6	1·6	2·3	1·6	2·3	1·6	2·3
„ „ „ 39	3·0	3·6	2·8	3·4	2·7	3·3	3·1	3·6	2·7	3·3	2·5	3·1	2·0	2·5	2·0	2·6	2·0	2·5
„ „ „ 60	3·4	4·2	3·2	3·9	3·1	3·7	3·4	4·1	3·1	3·8	3·0	3·5	2·3	3·0	2·3	3·1	2·3	3·0

Electrolytes used	10 Formic acid		11 Acetic acid		12 Propionic acid		13 Oxalic acid		14 Malonic acid		15 Succinic acid		16 Citric acid		Control	
Gels	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
Time in hours 6	1·1	1·3	0·9	1·1	0·8	1·0	1·3	1·6	1·1	1·4	1·0	1·3	1·5	1·7	0·9	1·1
„ „ „ 9	1·3	1·7	1·1	1·4	1·0	1·3	1·6	2·0	1·4	1·7	1·3	1·6	1·7	2·0	1·1	1·4
„ „ „ 15	1·6	2·0	1·4	1·8	1·2	1·6	2·1	2·4	1·9	2·3	1·7	2·2	2·1	2·6	1·4	1·8
„ „ 24	2·0	2·4	1·6	2·3	1·5	2·1	2·6	2·9	2·4	2·8	2·1	2·6	2·6	3·1	1·6	2·3
„ „ „ 39	2·3	2·8	2·1	2·6	1·9	2·4	2·9	3·5	2·7	3·3	2·5	3·2	3·2	3·6	2·0	2·5
„ „ „ 60	2·7	3·2	2·4	3·1	2·2	2·8	3·3	4·0	3·0	3·8	3·9	3·6	3·5	4·1	2·3	3·0

TABLE IV

Penetration of plasmoquine and quinine (base) (in cm)

Electrolytes used	1 NaOH		2 Na_2CO_3		3 $NaHCO_3$		4 HCl		5 HNO_3		6 H_2SO_4		7 $NaNO_3$		8 KNO_3		9 $Ba(NO_3)_2$	
Gels	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
Time in hours 6	14	17	10	14	09	13	13	16	09	13	08	12	08	10	08	10	08	10
„ „ „ 9	16	20	13	18	12	17	15	19	12	17	11	15	10	13	10	13	10	13
„ „ „ 15	20	25	18	23	16	21	19	24	17	22	16	20	12	17	13	17	12	17
„ „ „ 24	24	30	22	27	21	27	23	29	21	25	20	24	15	21	16	21	15	21
„ „ 39	29	35	26	32	26	32	28	34	24	30	23	29	19	24	20	24	19	24
„ „ „ 60	32	40	30	37	30	35	31	39	28	35	27	31	22	29	23	29	22	29

Electrolytes used	10 Formic acid		11 Acetic acid		12 Propionic acid		13 Oxalic acid		14 Malonic acid		15 Succinic acid		16 Citric acid		Control	
Gels	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
Time in hours 6	10	12	08	10	07	09	12	15	10	13	09	12	11	16	08	10
„ „ „ 9	12	16	10	13	09	12	14	18	12	16	12	15	16	19	10	13
„ „ „ 15	15	19	13	17	11	15	19	23	17	21	16	20	20	24	12	17
„ „ „ 24	18	23	16	21	14	20	24	28	23	27	21	25	23	30	15	21
„ „ „ 39	22	27	20	24	18	23	28	34	26	32	24	30	28	31	19	24
„ „ „ 60	24	31	23	29	21	27	32	38	29	35	28	34	31	39	22	29

TABLE V

Penetration of plasmoquine and cinchonidine sulphate (in cm)

Electrolytes used	1 NaOH		2 Na_2CO_3		3 $NaHCO_3$		4 HCl		5 HNO_2		6 H_2SO_4		7 $NaNO_2$		8 KNO_3		9 $Ba(NO_3)_2$	
Gels	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
Time in hours 6	1·2	1·5	1·0	1·3	0·7	1·1	1·2	1·5	1·1	1·4	1·0	1·3	0·7	0·9	0·7	0·9	0·7	0·9
„ „ „ 9	1·5	1·8	1·2	1·6	1·0	1·4	1·5	1·8	1·4	1·8	1·3	1·7	1·0	1·2	1·0	1·2	1·0	1·2
„ „ „ 15	1·8	2·3	1·7	2·0	1·5	1·8	1·8	2·2	1·7	2·1	1·6	2·0	1·2	1·6	1·2	1·6	1·2	1·6
„ „ „ 24	2·2	2·8	2·1	2·5	2·0	2·3	2·1	2·7	2·0	2·6	1·9	2·5	1·4	1·9	1·5	1·9	1·4	1·9
„ „ „ 39	2·6	3·3	2·5	3·0	2·3	2·7	2·5	3·2	2·4	3·0	2·3	2·9	1·8	2·3	1·9	2·3	1·8	2·3
„ „ „ 60	3·0	3·8	2·8	3·5	2·6	3·2	2·9	3·7	2·8	3·5	2·7	3·2	2·1	2·7	2·2	2·7	2·1	2·7

Electrolytes used	10 Formic acid		11 Acetic acid		12 Propionic acid		13 Oxalic acid		14 Malonic acid		15 Succinic acid		16 Citric acid		Control	
Gels	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
Time in hours 6	1·0	1·2	0·7	1·0	0·6	0·8	1·1	1·3	1·0	1·2	0·9	1·1	1·2	1·5	0·7	0·9
„ „ „ 9	1·2	1·5	1·0	1·3	0·9	1·1	1·3	1·7	1·2	1·5	1·0	1·4	1·4	1·7	1·0	1·2
„ „ „ 15	1·4	1·8	1·2	1·7	1·1	1·5	1·6	2·0	1·5	1·8	1·4	1·7	1·8	2·2	1·2	1·6
„ „ „ 24	1·9	2·2	1·5	1·9	1·3	1·8	1·9	2·3	1·7	2·1	1·6	2·0	2·1	2·8	1·4	1·9
„ „ „ 39	2·1	2·7	1·8	2·3	1·7	2·2	2·2	2·7	2·0	2·5	1·9	2·4	2·6	3·2	1·8	2·3
„ „ „ 60	2·5	3·1	2·2	2·7	2·0	2·6	2·5	3·1	2·4	3·0	2·2	2·9	3·0	3·7	2·1	2·7

TABLE VI

Penetration of plasmoquine and quinidine (base) (in cm)

Electrolytes used	1 NaOH		2 Na_2CO_3		3 $NaHCO_3$		4 HCl		5 HNO_3		6 H_2SO_4		7 $NaNO_3$		8 KNO_3		9 $Ba(NO_3)_2$	
Gels	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
Time in hours 6	1·0	1·2	0·8	1·1	0·7	1·0	1·0	1·3	0·9	1·2	0·8	1·0	0·5	0·7	0·5	0·7	0·5	0·7
„ „ „ 9	1·2	1·6	1·0	1·4	0·9	1·3	1·2	1·7	1·1	1·6	1·0	1·5	0·7	1·0	0·7	1·0	0·7	1·0
„ „ „ 15	1·6	2·0	1·4	1·8	1·3	1·7	1·5	2·1	1·4	2·0	1·3	1·9	1·0	1·3	1·0	1·3	1·0	1·3
„ „ „ 24	2·0	2·5	1·8	2·3	1·7	2·1	1·9	2·6	1·8	2·5	1·7	2·1	1·2	1·6	1·2	1·6	1·2	1·6
„ „ „ 39	2·4	3·0	2·3	2·8	2·2	2·6	2·4	3·1	2·2	2·8	2·1	2·7	1·5	1·9	1·6	1·9	1·5	1·9
„ „ „ 60	2·7	3·5	2·6	3·2	2·4	2·9	2·7	3·5	2·5	3·3	2·3	3·1	1·8	2·2	1·9	2·2	1·8	2·1

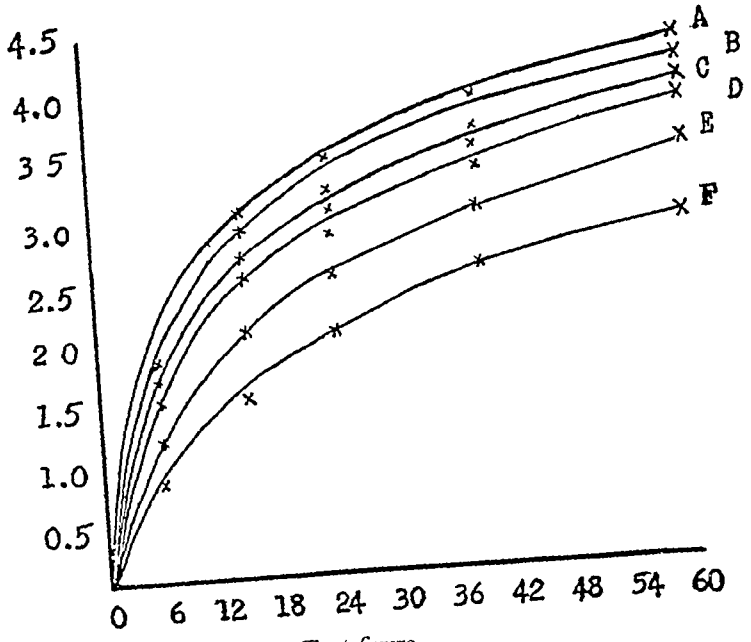
Electrolytes used	10 Formic acid		11 Acetic acid		12 Propionic acid		13 Oxalic acid		14 Malonic acid		15 Succinic acid		16 Citric acid		Control	
Gels	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
Time in hours 6	0·8	1·0	0·5	0·7	0·4	0·6	0·9	1·2	0·8	1·1	0·7	1·0	1·0	1·2	0·5	0·7
„ „ „ 9	0·9	1·3	0·7	1·0	0·5	0·8	1·1	1·5	1·0	1·1	0·9	1·3	1·2	1·6	0·7	1·0
„ „ „ 15	1·2	1·6	1·0	1·3	0·7	1·2	1·4	1·8	1·3	1·7	1·2	1·5	1·5	1·9	1·0	1·3
„ „ „ 24	1·5	1·9	1·3	1·7	1·1	1·5	1·7	2·1	1·6	2·0	1·5	1·9	1·9	2·4	1·2	1·6
„ „ „ 39	1·9	2·2	1·5	2·0	1·4	1·8	2·0	2·5	1·9	2·4	1·8	2·3	2·3	2·9	1·5	1·9
„ „ „ 60	2·3	2·5	1·8	2·3	1·7	2·1	2·2	2·9	2·1	2·8	2·0	2·6	2·6	3·1	1·8	2·2

(*iii*) plasmoquine and quinine (base),

(*iv*) plasmoquine and cinchonidine sulphate,

(*v*) plasmoquine and quinidine (base)

This behaviour can be shown graphically by the curves —



Text-figure

Curves A, B, C, D and E represent the penetration of cinchonine chloride, quinine bihydrochloride, quinine (base), cinchonidine sulphate and quinidine respectively in combination with plasmoquine in the presence of sodium hydroxide, while F represents that of plasmoquine alone

These curves can be mathematically expressed by the equation

$$P^2 = aT$$

where P is the penetration of the alkaloid in centimetres, T the time in hours and a the latus rectum of the graph

The experimental observations also show that the degree of diffusion of the mixture of quinine and plasmoquine is much more than what could be expected according to the Mixture Law In order, therefore, to see whether this law is followed with regard to some other important properties of this mixture of alkaloids, it was thought advisable to study the refractive indices of each of the drugs alone as well as in combination with each other Cinchonine chloride was selected for these experiments and the refractive

indices were measured at 20·07°C with the Abbe's Refractometer Four concentrations of this alkaloid gave the following results —

Concentration of cinchonine chloride	Ref. Index of cinchonine alone	Ref. Index of 1 per cent plasmoquine alone	Observed R. I. of plasmoquine and cinchonine	Calculated R. I. of plasmoquine and cinchonine
2·5 per cent	1·3302	1·3241	1·3271	1·32715
5 ,	1·3357	1·3241	1·3299	1·3299
10 ,	1·3431	1·3241	1·3336	1·3336
15 ,	1·3507	1·3241	1·3371	1·3374

This shows that the observed and calculated values are alike and the refractive indices of the mixture are in agreement with the Law of Mixtures

It has been shown in a previous paper that the degree of penetration of the alkaloid salts through the gel media may have some correlation with their therapeutic properties in the treatment of malaria From the experiments analysed above it is clear that the alkaloid salts in combination with plasmoquine penetrate much more quickly than when tried alone In fact the extent of penetration of this combination is very much in excess of what could be expected according to the Mixture Law The better clinical effects recorded after treatment with the combination of plasmoquine and quinine might, therefore, be due to the quicker and greater penetration of the two drugs either by enhanced absorption or increased parasiticidal action

The above experiments further indicate that whereas the penetration of the mixture of plasmoquine and quinine is considerably greater than either of them alone, that of plasmoquine when combined with cinchonine chloride is still greater This would suggest that if in this combination quinine is replaced by cinchonine chloride, a better medicinal effect might be produced This seems worthy of trial and such a treatment, besides other considerations, might also be more economical

Summary

The penetration of the important cinchona alkaloids into the chemical analogues of living cells has been studied in combination with plasmoquine It was found that —

(*a*) The effect of the presence of electrolytes in influencing the degree of penetration is related to their chemical nature

(*b*) Acids and alkalis accelerate the penetration while the neutral salts are generally without any effect

(*c*) The alkaloids penetrate much more when combined with plasmoquine than when tried alone

(*d*) The extent of penetration is very much in excess of what could be expected according to the Mixture Law

(e) The better clinical effects recorded in malaria after treatment with plasmoquine and quinine might be due to the greater and quicker penetration of the two drugs either by enhanced absorption or increased parasiticidal action

(f) Cinchonine chloride in combination with plasmoquine penetrates more than quinine and if penetration is any index of their clinical value, the drug is worthy of a trial

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COCAINE HABIT IN INDIA

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HISTORICAL AND GENERAL

COCAINE addiction is of comparatively recent origin in India. Inquiries in all provinces show that the drug was first used for its euphoric effects in Bhagalpur nearly 40 years ago and from there it came to Calcutta and spread to other places. The use of coca leaf for euphoric purposes, however, started many centuries ago in South America, the natives of Peru and Bolivia were known to indulge in the leaves of *Erythroxylon coca* as early as the 15th century. They were in the habit of chewing leaves during the times of great physical strain such as long laborious marches in the hills, as by so doing they felt refreshed and invigorated. Franz Pizarro in 1533 found that the leaves of this plant were used as an article of common luxury in Peru. There is a very interesting narrative of the trade in this leaf which was going on at that time with various tribes of Red Indians. According to tradition when the Empire of Inka was first established the children of the Sun offered the people coca leaves, which appeased hunger, imparted vigour into the tired and fatigued and made the sorrowful forget their sorrows, as a divine gift for their use. This plant with its wonderful properties was regarded by the people as an emblem of kingship so much so that the queen named herself 'Mama coca' after it. The priests also extolled its divine qualities and made use of it on

ceremonial occasions and the people of rank and power showed their divine nature by filling their mouths with these leaves and chewing them It was from them that these leaves found their way to the common folk who not only used them on ceremonial occasions but took them at all times for the exhilarating and pleasure giving effects to both the mind and body Their use therefore rapidly spread and evil effects began to manifest themselves among the people As long ago as the middle of the 16th century the ravages produced by the leaf were appreciated by the authorities and the second council of Lima tried to stop its use altogether among the inhabitants of Peru, Chile and Bolivia though unsuccessfully On the other hand the habit spread more and more and became deeply rooted among the populace with the result that moral, mental and physical degeneration set in all round Excessive chewing of coca leaf produced a state of hallucinatory confusion and delusion among the people often accompanied by digestive disturbances and marasmus Fortunately the alkaloid content of the leaf is small (1 0 per cent) and the harmful effects take longer to appear than when the pure alkaloid is used

The leaf was generally taken mixed with lime or ash of some plant The powdered leaves were kept in flask-shaped pumpkin shells and were taken out in small quantities with a needle the end of which was moistened in the mouth There were a number of other preparations also made from the leaf which were used by the populace The planters and miners of the land encouraged its use because they could get greater quantity of work out of the labourers under its influence The traveller Tschudi, who visited these parts about 1838, was so struck by its effects that he strongly recommended this custom to the European navies for long and fatiguing sailings of his day According to Johnston in the decade 1870–80, 15 million kilograms of dry coca leaves were produced and chewed by a population of 10 million souls Efforts to introduce coca leaf chewing into Europe, however, did not meet with much success Although the alkaloid cocaine was discovered in 1859-60, the importance of the plant from the medicinal point of view grew more from 1884 and the export of dry leaves from South America started from that time In order to reduce the cost of transport factories were started in Peru about 1890 which manufactured crude cocaine for export to other parts of the world During the year 1890, 1,730 kilograms of the crude alkaloid were exported and this increased to 10,600 kilograms in 1901 It was in this way that the alkaloid replaced the leaves and the knowledge of the effects produced by it spread to other parts of the world Between 1890 and 1900 cocaine began to be fairly largely used in the United States for euphoric purposes and the habit was also getting known to Europe, India and China It was thought at that time that administration of cocaine cured the morphia habit and alcoholism and this gave a stimulus to its use by the medical profession in the treatment of these conditions Unfortunately instead of curing morphinism it produced among many patients morphino-cocainism The successful use of the drug for producing local anæsthesia began to be appreciated more and more by

medical men, and this increased the demand for the alkaloid to such an extent that it was considered worth while to produce it by synthetic methods The preparation of the alkaloid, however, is easier and cheaper from the leaf, and large plantations were started in Java and other places The world thus became independent of South America, and the alkaloid became comparatively cheaper in price The leaf from Java goes to factories in Europe, America and Japan and the South American product has been practically driven out of the market In 1922, 1¼ million kilograms were exported from that island with a cocaine content of 1 5 per cent

It was about 1900 that the use of cocaine for euphoric purposes spread extensively in the United States and began to make its way into India among other places In 1902 the first report appeared about the use of cocaine in form of snuff by negroes, and many cases of ulceration of septum of the nose were recorded as a result of this During the Great War, there was such an enormous spread of the habit in Europe and particularly in France, that vigorous preventive measures were taken by authorities to stop its use

Erythroxylon coca does not grow in India in a state of nature No reference is to be found in the ancient Sanskrit literature about the leaves or the properties possessed by them The only references in the medical literature that exist are of very recent origin and concern the use of the alkaloid cocaine and not the leaf The variety *Erythroxylon monogynum* is said to be indigenous to India (Madras) but it does not contain cocaine or the related alkaloids This variety has smaller leaves than *E coca,* can stand higher temperature and it is thought that if cultivated under proper conditions it may produce cocaine Two varieties of Erythroxylon are believed to grow wild in Bombay Presidency but if so they are extremely rare One of them yields a mere trace of cocaine, the other does not yield any alkaloid at all The habit of chewing coca leaves consequently never existed in India

In 1870 *E coca* was brought to Ceylon from the Botanical Gardens at Kew (London) and in 1885 it was brought over to British India The leaves when carefully cultivated under suitable conditions have been found to be rich in cocaine, the yield increasing with the age of the plant It is not quite certain if the cultivation would be a commercial success as it has been in the Dutch Indies in Java where the trees scientifically cultivated give a better yield of the alkaloid than those produced in South America In India it was noted that the best results were obtained from the plant grown on the uplands of the Nilgiri Hills and those planted in the hot low plains did not thrive and died out

E coca has, however never been cultivated in this country on a large scale Some years ago (1926) it was suggested in the English daily press in India that cocaine bearing *Erythroxylon coca* was growing wild all over the country, that the people were learning the habit of chewing the coca leaf, and that there might be secret factories for manufacture of cocaine In support of this theory it was argued that large quantities of the drug were seized

on railways and the cocaine habit was spreading rapidly and no one had been able to trace the source from which the drug was obtained The alleged cultivation of coca plant was also referred to at a meeting of the advisory committee of the League of Nations on traffic in opium and other dangerous drugs in 1925 Careful inquiries were then made by the Government of India and recently we have been able to fully corroborate the views then expressed by the authorities Neither *Erythroxylon coca* nor any other plant from which cocaine can be produced is cultivated in India, except that *E coca* is sometimes grown as an ornamental plant in the gardens in Bombay and there are specimens at the Royal Botanic Gardens, Calcutta, and in the Botanical Gardens at Madras and Kallar (in the Madras Presidency) *E coca* so far from growing wild all over the country is not known to grow wild anywhere in India A few plants were found in some of the Nilgiri estates, which were in all probability the relics of the experiment made in 1885, but even these contained little or no cocaine The manufacture of cocaine is a highly technical process and there is no ground whatever for the belief that cocaine is secretly manufactured in India and as will be shown in subsequent pages there is no mystery whatsoever about the source of the illicit cocaine seized in India It is undoubtedly all manufactured in certain countries outside India

Cocaine habit in India

As early as the nineties of the last century it was realized that cocaine was being used in certain parts of the province of Bengal and Bihar for its euphoric effects The earliest record of its use came from a small town named Bhagalpur The story is related of a big land owner of that place who contracted the habit accidentally after its use to relieve dental pain So extraordinary were the effects produced on him that not only did he become habituated to its daily use but passed on the habit to many others It was stated at that time that cocaine was secretly sold to a considerable extent to school boys and students, merchants and men of good class in the community The price of the alkaloid at that time was Rs 3 per drachm or about one anna per grain, and it was usually sold to the public in packets of ½ grain each The evil effects produced by the drug were not fully appreciated at that time by the profession and the laity and therefore no restrictions were imposed on the sale and use of this dangerous drug

The habit, however, spread so quickly from Bhagalpur to Calcutta and other large towns and the ravages produced by it in the addicts became so evident in a short time that it soon came to the notice of the medical profession and the authorities Steps were at once taken by the Excise Department to restrict its import and scale In the meantime, unfortunately the evil had taken root and many large towns had become affected The habit had spread along the two main routes even to Northern India It worked its way up through Benares, Lucknow, Rampur, Saharanpur and Ambala on the one side, and through Allahabad, Cawnpore, Agra, Muttra and Delhi on the other side

We were credibly informed that in Delhi the addiction existed on a fairly extensive scale in the year 1900 In this town it is reputed to have spread through the agency of a medical practitioner who prescribed it as a stimulant and as a tonic In Saharanpur the habit was fairly common 20 to 25 years ago, and there a trained midwife is said to have been responsible for its introduction Tracing its progress further north there is no doubt that the spread of the habit to the town of Amritsar in the Punjab was through shawl merchants, who were in constant communication with Calcutta From Amritsar the addiction spread to Lahore Peshawar was also involved early owing to large number of inhabitants of this town being constantly on visits to Calcutta in connection with the fruit trade A very able excise officer of the North-West Frontier Province assured the senior author that Peshawaris were in a great measure responsible for trafficking in cocaine carried on in India Large quantities of Charas (resin of *cannabis sativa* manufactured in Central Asia) were smuggled through the North-West Frontier Province and sold at a very cheap price along the frontier These were carried by them to such big centres as Bombay and Calcutta and were sold at very large profits The proceeds of this sale were employed to smuggle cocaine back from the sea-port towns to different parts of India particularly large towns of Northern India He was certain that the key to the smuggling of cocaine lay in the North-West Frontier Province With the reduction of duty on 'Charas' by 30 per cent which has recently been enforced, this smuggling has already been brought down to a very great extent and this will undoubtedly react on trafficking in cocaine

Control over the import and sale of cocaine

In the early days of cocaine in India there were no restrictions on its import, sale or use It did not take long for the authorities to realize the seriousness of the problem and regulations were brought into force to strictly control the sale of this dangerous drug Not only was the alkaloid forbidden to be sold to the general public but the sale by druggists and chemists to medical practitioners and dentists was also strictly controlled No one is allowed to possess coca leaf, alkaloids of coca or any preparations made from them, or preparations containing ecgonin or any substance chemically allied to cocaine or having similar physiological effects, except under a special licence Even possession of these drugs by licence is strictly limited In spite of this, writing in 1913 in the *British Medical Journal,* Dr Chuni Lall Bose said 'In Calcutta, despite the vigilance of the excise authorities and notwithstanding the stringent measures adopted by the Government against the possession and sale of this substance by unlicensed persons, there is reason to believe that the cocaine habit has much increased and is rapidly spreading'

The present extent of cocaine habit in India

It is not possible to say with any degree of accuracy the present extent of cocaine habit in India Tuke (1914) said that the habit of taking cocaine

was by no means confined to the poor and uneducated classes From the information we have gathered from our work in the field in various provinces of India, it transpires that only members of the medical profession at first knew about the euphoric properties of cocaine and that it was from them that the lay people learnt about its effects As in early days there were no restrictions regarding the possession and sale of the alkaloid, the habit quickly spread from one commercial city to another on account of the more rapid methods of transport which were coming into vogue owing to the extension of the railway system in the earlier part of this century The stimulant effects produced by the drug were a great attraction to a type of individual, who was ignorant of its evil effects on the system Moreover the enormous financial gain which the dealers in this nefarious traffic obtained, soon induced them to employ agents to push on their trade and to advocate and popularize its use It thus came about that, even when restrictions were imposed the use of the drug was not curtailed but rather spread so much so that cocaine to-day is a well-known commodity to many of the inhabitants of large towns in India It is popularly believed to be a sexual stimulant, and many start it for this purpose The other attraction for its use is that it has a most extraordinary effect, temporary though it be, in rapidly overcoming mental as well as physical fatigue As we have already stated, its use rapidly spread from Calcutta to large towns along the two main railway routes through the United Provinces into the Punjab and to the North-West Frontier Province and even to the tribal territory on the North-West Frontier of India The drug was also smuggled into Bombay and on that side its use spread to different large cities of the Bombay Presidency (e g, Ahmedabad), Central India and the Central Provinces We have been impressed by the fact that it was the large towns along the main railway lines from Calcutta and Bombay which were affected Large cities along the branch lines remained free from this addiction or were only affected in exceptional cases The only part of India where the habit seems not to be known to any extent is the Madras Presidency

It is very difficult to form any accurate estimate of the prevalence of addiction to cocaine in different towns, for the habit of taking this drug is generally considered to be so disgraceful by the people that no self-respecting person will own it Besides this both the sale as well as the possession of cocaine is illegal, and the possessor of the drug as well as the dealers are liable to prosecution and punishment under the Dangerous Drugs Act Our work in the field was therefore very difficult and delicate and sometimes dangerous After several years of patient work we have been able to form some idea of the prevalence of this habit among the people of India We are specially grateful to the excise authorities for their help and co-operation in this work But for the personal interest many of them took in this research it would have been difficult for us to make any headway We were surprised to find that not only is the habit getting hold of the ignorant masses in the lower strata of society but some of the wealthy families with education and culture are

also falling a prey to this drug The addiction in fact is spreading among the female members of many of the well-to-do families particularly in the United Provinces and to a lesser extent in the Punjab and in Gujrat The dealers in this drug fully realize that unless they get hold of the wealthy people who can afford to pay a high price for the drug, it would not be worth their while to carry on this rather hazardous trade They therefore do their best to introduce the drug into the homes of rich people through the agency chiefly of betel leaf sellers and servants The most popular way of taking the drug in India is in a betel leaf and it is quite an easy matter for a designing person to put the drug in a 'pan' and introduce it into the household of unsuspecting individuals who become so impressed by its extraordinary effects that not infrequently the habit is acquired without realizing its dangers It is also for this reason that addiction to this drug is more prevalent among the section of population who are in the habit of chewing betel leaf

We have tried to form an idea of the prevalence of the habit in India by careful inquiries in the towns where the habit is known to exist Although it is not possible to make an accurate estimate of the prevalence of this addiction, we can form some idea of its distribution among the populace The excise authorities know the persons who are carrying on this traffic and in various provinces, there being printed lists of those engaged in trafficking in cocaine The authorities are powerless to put an end to their activities, because of the great difficulty of proving the offence in a court of law Many of the persons dealing in this trade are dangerous individuals, and people are afraid to come forward and give evidence against them and more often than not the cases brought against these persons fail

In the following map we have roughly depicted the information we have obtained with regard to the prevalence of cocaine habit in India

We have grouped the different towns under four classes In class (1) are included towns in which the addiction is very prevalent 0 5 to 1 per cent of the population taking it In class (2) are given those towns in which the addiction is fairly prevalent, i e , somewhere between 0 25 to 0 5 per cent of the total population In class (3) are included places where addiction is in existence but to a very small extent For instance in a town of 20 to 40 thousand souls there may be 10 to 20 addicts In class (4) are included those towns in which the habit is just being introduced The smaller and larger distributing centres are also depicted in the map

A perusal of the map will show that the worst province so far as cocaine addiction is concerned is the United Provinces where the chief centres of addiction are in the following towns Lucknow, Saharanpur, Benares, Agra, Allahabad, Aligarh, Bareilly, Moradabad, Rampur State, Meerut and Cawnpore come under the class (1), Ghaziabad, Muttra, Mirzapur and Furrukhabad in class (2) Jaunpur, Fatehpur and Dehra Dun in class (3) There are a large number of smaller towns in class (4) into which the habit is just being

Rangoon are received from Amoy by Chinese-owned Hong Line of steamers plying between Amoy and Rangoon China itself manufactures no cocaine but imports it from Japan and Europe A consignment made in Burma in 1928 included some items bearing the name of a reputed firm in Germany, that were traced to a consignment sent to a Chinese firm of chemists at Amoy Most of the cocaine which is being received bears labels which are entirely fictitious The commonest met with is that of Fujitsuru brand showing a stork in flight with a mountain in the back ground From the information which it has been possible to gather in this country it appears that this brand is packed and labelled and received by the Calcutta-bound carriers in Japan, but that a copy of the label of this brand is made use of by dealers in Amoy who put up an adulterated product for despatch from there to Rangoon Other fictitious brands found are the Elephant, Buddha, K s, and Tacmnura As there is no line of steamers that comes direct to India from Japan without calling at a Chinese port, it cannot be proved absolutely that the source is Japan and not in China Japan, however, is known to have factories but China has none On the other hand China may get its supply from Europe Side by side with these fictitious brands, cocaine is also found bearing the labels of genuine Japanese factories namely the Hoshi, Koto Seiyaku Takeda and Sankyo firms, and it is an interesting fact that cocaine found with Japanese smugglers in this country—for there are Japanese engaged in the Indian import traffic too—bear either these genuine labels or none at all, any way not those of the Fujitsuru, Elephant, etc As far as can be ascertained, Amoy supplies Burma and no other place, Calcutta supplies come from Japan, either direct or transhipped at Hongkong or Singapore The drug is brought in hidden and in some cases even the officers of the ship have been implicated, it is stowed away in all sorts of inaccessible places in the boats On account of its small bulk the landing of the drug does not appear to present much difficulty to those engaged in the traffic It is often not brought into the port at all and is thrown overboard in watertight packets int a or into the river from where it is picked up by a very organized smugglers way large quantities of the drug find then way s like Calc the hands of large dealers

The amount seized by the Calcutta c last ye 0 ounces and experienced officers place the een 2 t of the quantity actually got through, this somewhe to 250,000 ounces of cocaine was successfully s the co been calculate mpetent authorities th in Ind paid between hs and Rs 648 lak lers during 1929 enormous sum of idea of the tot f persons habitua Taking an aver 2 to 3 grams between a quarter million individ its euphoric effects is very m

amount of cocaine smuggled is heavily adulterated by the dealers in this country

It is not necessary for us to go into the details of the devices specially employed by the smugglers These have been dealt with in some detail in a pamphlet by Mr A T Bhargava of the Criminal Investigation Department in a pamphlet published by the Oriental Press of Allahabad in 1916 and can be added to indefinitely It will be sufficient here to say that many extraordinary and novel methods are used which enables large quantities of the drug to be smuggled through into the hands of organized gangs in centres like Calcutta and from there to different towns up-country There is in existence a very elaborate system of the distribution of the drug from the port of import to the remotest towns in India Depots have been established by the traffickers in towns where there is demand from which the retailers know how to get their supplies Ordinary people in the guise of menials, such as grass-cutters cobblers or labourers whom nobody would even suspect are usually employed for bringing the drug Sometimes purdah women in a 'burka' act as carriers Even respectable looking Anglo-Indians and Europeans travelling in 1st and 2nd class in the railways have been found in possession of large quantities of cocaine The distribution to the addicts is effected chiefly through the agency of 'pan' or betel leaf sellers as well as through wandering pedlars with no fixed abode

We have stated that the drug smuggled into the country is not infrequently already adulterated The retail dealers further adulterate it with cheaper similar looking products such as phenozone, acetyl salicylas potassium nitrate, etc, the last named because it imparts a sense of coolness to the tongue somewhat resembling cocaine One consignment recently captured in Delhi consisted of pure phenozone The drug is then made into small packets or 'fafa' containing ½ to 3 grains each and is handed over to the pedlars who are personally in touch with the addicts The police and excise authorities have made a large number of arrests and therefore these people are getting more careful As it is unlawful to possess or to sell cocaine, they do not carry it on their person They place the packets in some unfrequented place and indicate the situation to the addict after they have received the price The wholesale and retail dealers who keep stock of cocaine are to be found in different towns and in many cases are known to the police Actual prosecution and arrest are, however, very difficult because the traffickers are very clever in evading the law They have got effective methods of disposing of the drug at a moment's notice when their places are raided by the authorities The favourite method is to keep some water handy and as soon as a police raid is apprehended the drug is thrown into water dissolved and the solution poured into the nearest drain It thus comes about when the police enter the place and search they do not find any trace of the drug Carriers of cocaine from one place to another are very rarely caught by the excise authorities They carry small quantities which can be easily concealed on the person and when

bringing cocaine from big centres these people often do not disembark from the train at big stations where they are likely to meet excise guards They frequently get out in small suburban or wayside railway stations and convey it by road to large towns Even if some of them are arrested they are paid well enough to go to jail and not to give the chief culprits away Very often after doing a few months hard labour they come out and resume their trade Thus the real persons who manage the business of smuggling cocaine on a large scale not only escape but grow very rich by this nefarious trade

The betel leaf seller and women of low morals such as prostitutes play an important part in the spread of the habit to their clients They are sometimes the medium through which the drug is sold and they induce their customers to buy it and indulge in it A number of our addicts attributed the beginning of the habit to their association with these women

An analytical study of 200 cases of cocaine addiction

These cases were not collected by any selection, the addicts were examined, the histories and symptoms were recorded as they were met Majority of the cocaine eaters hailed from Delhi northern towns of the United Provinces and southern towns of the Punjab The addicts were not seen only once but with many of them, especially those from Delhi, it was possible to maintain contact for months so that a more detailed and thorough study was possible It may be mentioned here, however, that they all hailed from the lower strata of society such as artisan and menial class For obvious reason we could not get any of the better class people who indulge in the drug to come forward for examination We met a number of them, however, and are able to say that better hygienic conditions, good food, etc, did not make much difference as regards the symptoms and physical effects produced by the drug In fact we found that wealthier people indulged in larger quantities of the drug and in them the process of physical, mental and moral degeneration progressed more rapidly

Race and religion—The following is the division of the cases according to the religion

		Percentage
Mahommedans	125	62 5
Hindus	68	34 0
Christians	4	2 0
European	3	1 5
Total	200	100 0

It will be seen that the majority of our cases were Mahommedans (62 5 per cent) and next come the Hindus (34 0 per cent) Our impression of other

parts of India also is that proportion of Mahommedans taking the drug was considerably higher than the Hindus in the class of case we were able to study

Causation of cocaine addiction—A perusal of Table I will show that 55 5 per cent of the addicts contracted the habit by association with other addicts and 20 5 per cent of these by association with women, 21 per cent were well-to-do people who took the drug as luxury and for pleasure, 10 5 per cent were actually carrying on traffic in the drug, 6 5 per cent took it for worry and fatigue, and 4 5 per cent out of mere curiosity In only 4 per cent was there any history of disease, which is a great contrast to the causation of the habit in western countries In many cases the history given was that they were introduced to the drug by persons carrying on the traffic

TABLE I

Showing causation of cocaine addiction as worked out from a series of 200 *addicts*

	Association		Luxury and pleasure	Traffic in cocaine	Fatigue, worry, etc	Curiosity and fancy	Disease	Total
	Association with males	Association with females						
Number of addicts	70	41	42	21	13	9	4	200
Percentage of addicts	35	20·5	21	10·5	6·5	4 5	2	100

Duration of addictions—Table II gives the important information as to how long the habit of taking cocaine has been going on in India It will be seen that in only two cases was the habit contracted over 40 years ago, 99 0 per cent having used the drug for less than 40 years The use of cocaine, it would appear, was first started not more than 40 years ago in Northern India, 45 years ago the drug was unknown there This table also brings out the interesting fact that cocaine habit has been rapidly increasing in this country of late years It will be seen that 64 0 per cent of our cases started it during the last 10 years, 27 5 in the previous decade and 13 5 per cent in the decade previous to that It will be noticed that the increase during the last ten years is very considerable This table also brings out the interesting fact that once the habit is formed, it is difficult to break off and in the majority of cases it is continued for long periods

TABLE II

Showing the duration of addiction as worked out in a series of 200 cocaine addicts

	Duration in years					
	1–10 years	11–20 years	21–30 years	31–40 years	41–50 years	51–60 year
Number of addicts	128	55	27	8	2	
Percentage of addicts	64·0	27·5	13·5	4·0	1·0	Nil

Table III gives some interesting facts The commonest age of contracting the habit is between 15 and 30 years of age In as many as 19 per cent the habit was started early between the ages of 10 and 15 years, showing that the drug was conveyed to the boys either through the agency of pedlars or through servants in well-to-do families The vast majority of the addicts (70·5 per cent) started the habit between the ages of 16 and 30 more than half of this number being between 16 and 20 years This table brings out the fact that cocaine habit in India is chiefly a habit of the 2nd or 3rd decade of life when the sexual functions are at their highest and sexual instinct dominates In a small minority only the habit was started in later life

TABLE III

Showing ages at which the addiction was started in a series of 200 cocaine addicts

Years of age	Ages of the addicts at which the habit was started									
	5–10 years	11–15 years	16–20 years	21–25 years	26–30 years	31–35 years	36–40 years	41–45 years	46–50 years	51 and above
Number of addicts	6	32	72	41	28	9	4	3	4	1
Percentage of addicts	3·0	16·0	36·0	20·5	14·0	4·5	2·0	1·5	2·0	0·5

Present age of addicts—A perusal of Table IV shows that no cases occurred before the age of 15 years This is not due to the fact that the habit is not started before that age, but because for obvious reasons such addicts would not come forward for examination Between the ages of 16 and 40 years the incidence was 77·5 per cent, between 40 and 50 years 13·0 per cent, after

50 years very few cases occurred It will be observed that 90 5 per cent of cases occurred between the ages of 16 and 40 years From this it would appear that cocaine habit in this country is an addiction chiefly of young age It begins with adolescence, reaches to maximum between 21 and 40 and then declines rapidly as age advances It is thus a habit confined mainly to the period of life at which the sex impulse is predominant, i e, the sexually active or romantic period of life This table further brings out the fact that indulgence in the drug adversely effects the longevity of the individual, probably many of the addicts living not much longer than 40 years

TABLE IV

Showing the present ages in a series of 200 cocaine addicts studied

Years of age	PRESENT AGES OF THE ADDICTS										
	16–20 years	21–25 years	26–30 years	31–35 years	36–40 years	41–45 years	46–50 years	51–55 years	56–60 years	61–65 years	66–70 years	71 and above
Number of addicts	18	23	48	34	32	13	13	5	5	4	2	3
Percentage of addicts	9 0	11 5	24 0	17 0	16 0	6 5	6 5	2 5	2 5	2 0	1 0	1 5

Occupation or vocation followed by the addicts—It will be seen that the majority of addicts are from the artisan class, shop-keepers, hackney carriage drivers and tradesmen, who make a modest living The rest came from practically all classes of people chiefly the menial class Many of these men earned Re 1-8 to Rs 2 per day, they spent only half or sometimes less than that for their food or for the maintenance of their families, the balance was spent on buying the drug

Table VI gives information regarding the existence of the habit in other members of addict's family, its prevalence among the married and unmarried individuals and sex incidence, the association of the habit with other intoxicant drugs, and daily dosage taken by the addicts

Heredity—As regards the existence of the habit among the parents and other members of the addict's family, it will be seen that in 192 cases out of 200 there was no history of addiction in the family, of the remaining eight in seven only one other member of the family took the drug and in the case of the remaining one more than one member was addicted The cocaine habit in India, however, has not lasted long enough for determining its effects on heredity

Marriage and fecundity—Matrimony as a factor appears to be of some importance in this addiction as more than 60 per cent of the addicts were

TABLE V

Showing occupation or vocation followed by a series of 200 cocaine addicts studied

Number	Occupation	Number of cases	Percentage
1	Artisan	50	25·0
2	No work	23	11·5
3	Shop-keeper	22	11·0
4	Cocaine seller	11	5·5
5	Tonga driver	10	5·0
6	Tradesman	8	4·0
7	Betel leaf seller	7	3·5
8	Labourer	7	3·5
9	Beggar	6	3·0
10	Stall-keeper	5	2·5
11	Prostitute	5	2·5
12	Musician	4	2·0
13	Clerk	4	2·0
14	Domestic servant	4	2·0
15	Mechanic	4	2·0
16	Butcher	4	2·0
17	Sweeper	4	2·0
18	Police pensioner	3	1·5
19	Agriculturist	3	1·5
20	Priest	2	1·0
21	Peon	2	1·0
22	Household woman	2	1·0
23	Confectioner	2	1·0
24	Excise informer	2	1·0
25	Wrestler	1	0·5
26	Painter	1	0·5
27	Jeweller	1	0·5
28	Compounder	1	0·5
29	Hakim	1	0·5
30	Landlord	1	0·5
	TOTAL	200	100·0

TABLE VI

Showing the effects of heredity, sex incidence, association with other drugs and dosage in a series of 200 cocaine addicts studied

	FAMILY HISTORY IN CASE OF 200 COCAINE ADDICTS			MARRIED OR SINGLE			SEX		HISTORY OF OTHER DRUG ADDICTION			DAILY DOSAGE				
	No family	With family history		Single	Married		Sex		History of other drugs habit							
	No family history	Of one relative	Of more than one relative		With issue	Without issue	Males	Females	No other drug habit history	Of one drug	Of more than one drug	1–2 grains	3–4 grains	5–10 grains	11–15 grains	16–25 grains and above
Number of addicts	960	7	1	122	58	20	193	7	125	45	30	90	43	48	8	11
Percentage of addicts	192	3·5	0·5	61·0	29·0	10·0	96·5	3·5	62·5	22·5	15·0	45·0	21·5	24·0	4·0	5·5

unmarried, which in a country like India is a high percentage Out of the 78 married individuals 45 had issues and 20 had none The series of cases is not large enough to enable us to draw any conclusion regarding the effect of the drug on the fecundity of the addicts

Sex incidence—In our series of 200 cases 193 were males and 7 females, of 7 females two were household women and 5 were prostitutes These low figures among females are explained by the fact that most of the women who take the drug would not come forward to be examined It does not mean that the incidence of the habit among women is so low We have personal knowledge that the habit is not uncommon among the female members of well-to-do families in the United Provinces and parts of the Punjab

Association of cocaine habit with other drugs—In 125 of our addicts there was no history of taking some other euphoric drug, 45 took one drug and 30 took more than one drugs Alcohol is taken by many of the cocainists, next comes opium and lastly *cannabis indica* preparations 'Charas' and 'Bhang' A person indulging in cocaine will not hesitate to take any other intoxicant drug if he cannot get his dose of the alkaloid

Dosage—A perusal of the Table will show that 45 per cent of the addict took doses of 1 to 2 grains daily, 21 5 per cent 3 to 4 grains, 24 per cent 5 to 10 grains and only 9 5 per cent took doses larger than 10 grains daily In considering the dosage it should be remembered that this apparent dosage does not by any means represent the amount of the alkaloid actually consumed Most of the cocaine sold by the retail dealers is very badly adulterated and it may be safely said that many of the addicts did not actually consume half or even a third of the quantity of the pure alkaloid stated to have been consumed The average dose of an addict from our series would work out to be roughly 2 to 3 grains of the adulterated drug daily

Ætiology of cocaine addiction

(1) *Association*—Cocaine habit in Europe and America during the last decade, has in the majority of cases been acquired after using the alkaloid as a medicine In India, however, the case is entirely different In this country the habit is to some extent confined to the class of individuals who is more or less addicted to other narcotic drugs such as opium, 'ganja' or alcohol, but it is often met with in people who are not using any other drugs The chief cause of the habit is association with other cocaine addicts or people who are taking other narcotic drugs It is a curious fact that people addicted to euphoric drugs always try and persuade their friends and acquaintances to try the drug of this choice We have come across cases where betel leaf with cocaine had been given to an individual without his knowledge at first and he afterwards became an addict Many of the older addicts told us that their first introduction to the drug was by the traffickers in cocaine who came to them and told them that they will give them something which will produce most wonderful sensations and effects They were given the first few doses

gratis and when they appreciated the effects they were so much attracted by the drug that they spent all their fortune in buying it and doping themselves with it

Others try the drug out of sheer curiosity Some of those belonging to the latter groups get such severe reactions after taking it that they will not repeat the experiment Others, however, generally the class of individuals whose mental equilibrium is not stable, get such charming and attractive effects, temporary though they be, that they almost invariably repeat the experiment at their own expense and in this way gradually become victims to the habit Young and inexperienced lads often become addicted and ruined in this way We have known many cases in which boys in the towns have contracted the habit through the agency of their menial servants or other designing persons

(2) *For its pleasure-giving effects and as a luxury*—Cocaine in India is more or less an addiction which is confined to people with licentious and vicious habits Sexual vice and dissipation are very prevalent among this class and they are always anxious to find new avenues of pleasure-giving sensation Some, especially those belonging to the indulgent rich class, take it because their senses of appreciating pleasure are tired and they want something to stimulate them so as to be able to appreciate pleasurable sensation to which they have become insensitive through long continued usage Such persons may be addicted to other drugs and are at the same time in search of new sensations This class is not uncommon in large towns even among the artisan class because the surroundings here are unhygienic and people take to drug habits for want of healthy recreative occupations There is also the idle rich class already referred to in towns who have nothing to do and who indulge in the use of euphoric drugs for the sake of having something to do and to make life worth living

(3) *For sexual gratification*—Cocaine is one of those drugs which are very frequently used by the women of the underworld and prostitutes not only for their own use but also for the licentious people who visit them, with the idea of giving them new pleasurable sensations and stimulating their sexual desires We will deal with the effect of this alkaloid on the sexual faculties later It will suffice here to say that the effect of the alkaloid is a temporary stimulation of the psychic areas, the mental excitement resulting from it giving a semblance of aphrodisiac effects in some individuals certainly in the female sex

(4) *Fatigue, worry and strain*—These are among the commonest causes of cocaine habit in the western countries, but such factors do not appear to play a very important part among the Indian addicts Cocaine undoubtedly removes the feeling of fatigue and hunger for the time being and gives a feeling of self-satisfaction and forgetfulness to the person, who generally takes it towards the evening after the day's work is over As the effect of one dose lasts for a short time only, the desire to repeat it at frequent intervals becomes

irresistible in these individuals and they only forego the next dose if its acquisition is beyond their means

(5) *Disease*—This is a very rare cause of the habit in this country although in western countries it accounts for a large number of addicts directly or indirectly In our series of 200 cases we only came across with 2 cases who started the habit because it relieved attacks of asthma from which they were suffering and for which they first took the drug

(6) *City life and cocaine addiction*—Cocaine addiction in India is chiefly confined to dwellers of towns and cities Insanitary condition, overcrowding, want of hygienic and healthful recreations, the strain and stress of life in large towns all predispose to addiction to euphoric drugs Cocaine addiction is practically unknown in the rural areas at the present time because the economic condition of the people does not allow them to spend their hard-earned money on such an expensive drug and partly because the habitue will at once be found out by others and will thus be disgraced

(7) *Nervous and psychological factors*—Cocaine habit, like narcotic drug habits, is chiefly confined to individuals whose psychic condition is in an unstable state of equilibrium Most of the addicts we examined could be put under two classes (*a*) weak-minded phlegmates and mentally dull and deficient individuals, there may be family history of insanity, alcoholism or neurosis in such cases These individuals are generally unable to stand the daily stress of life and often resort to drugs to enable them to carry on their daily routine Such people do not resort to a drug for sexual or vicious purposes but merely for its stimulating and euphoric actions We have pointed out elsewhere, that this is the common cause of opium habit in India and we are emphatic that this factor also plays a very important part in cocaine addiction in this country After the dose the patient who was irritable and depressed becomes self-confident and cheerful and able to face the world with fortitude (*b*) Irritable, nervous and hypersensitive temperaments These persons form a smaller group They become easily upset and are irritated by small worries and pin-pricks of life of which normal individuals take no notice They want some sort of a depressant or a narcotic which would make them forget their mental worry and give them a sense of restful sleep and a quiet life, or a stimulant which would give them apparent courage to face their troubles with fortitude We have met people who were of a very irritable and quarrelsome nature and who resorted to a drug for this reason Many such people under the effect of opium or *cannabis indica* become entirely changed

Methods of taking cocaine

After the isolation of the alkaloid the chief method of taking the drug in the western countries was by hypodermic injection and owing to difficulties of administration the habit did not spread to any great extent at that time Soon, however, the easy method was discovered of taking it in form of snuff

and by rubbing it on the gums This was quickly followed by spread of the habit to large centres of negro population in the United States

The most common method of taking cocaine in India is by putting it in 'pan' or betel leaf That is the reason why addiction to the drug is more prevalent amongst people who indulge in 'pan' eating As is well known the betel leaf is taken by mixing it with small quantities of catechu and slaked lime, a little betel nut or sometimes spices such as cinnamon, cardamom, ginger, etc, are also added The drug is either mixed with the spices and then wrapped in the betel leaf or some of the addicts place the alkaloid on the dorsum of the tongue and then chew a 'pan' immediately afterwards Addicts who have been indulging in the drug for a long time generally put the cocaine on the tongue and merely take a little lime and catechu afterwards dispensing with the betel leaf It is said that by doing this the action of the drug is enhanced and the effects produced are stronger Rarely the drug has been taken in form of a solution, obtained on a doctor's prescription, the addict sipping the solution at intervals following it each time with a betel leaf The method of rubbing the drug into the gums or taking it as a snuff is up to the present time unknown in this country A rare method which is sometimes used, particularly by the prostitutes, is that of injecting a solution of cocaine into the vagina by means of a douche can This gives the individual a sense of local constriction and the general systemic effects appear almost immediately The sexual act is said to be prolonged if the drug is administered in this way

Symptomatology of cocaine addiction

From a careful study of 200 cases which have been analysed above we have been able to form some idea of the symptoms and effects produced by cocaine on Indian addicts By getting personally in touch with the addicts we could get their own description of the symptoms which were produced after the drug was taken

Immediately after taking the drug there is a slight smarting or tingling sensation in the tongue, the lips feel swollen, dry and thick There is irritation in the fauces and sensation of constriction in the throat Soon there is a complete loss of sensation in the oral cavity, tongue and lips There is a feeling that the tongue is missing from the mouth After these preliminary sensations which last only a few minutes, the drug begins to gain entrance into the circulation and this is the beginning of the stimulant stage There is a slight feeling of dizziness or heaviness in the head, there is a throbbing sensation in the arteries of the neck and palpitation of the heart The unpleasant sensations of confinement and air hunger sometimes occurring after subcutaneous injections is not frequently met with when the drug is taken by the mouth unless the saliva is swallowed or enters the stomach by trickling through the œsophagus The pulse becomes slightly full and quick, but as a rule does not exceed 100 to 110 per minute There is a very pleasant feeling of heat

all over the body There is a feeling that something is being drawn away from the limbs towards the head and the mouth and a peculiar delicious feeling is perceptible in the region of the tongue The ears become hot and red, the cheeks become pale, the tip of the nose becomes cold, and the patient begins to perspire on the forehead and over the neck These symptoms are more common in the early stages of addiction, but are hardly perceptible in confirmed addicts of long standing unless they take an overdose

By this time a peculiar sensation of excitement is felt by the individual, he feels cheerful and has a sensation of comfort both in the mind and the body He feels capable of undertaking anything however difficult, whether it may involve physical or mental effort During this period the patient looks very keen and excited, his eyes are bright and he talks coherently Complicated intellectual work may be done during this period without mistakes The increased sensitiveness of the sense nerves makes proper conception of nerve impression possible The addict gets agreeable hallucinations, he imagines himself to be a wealthy man such as a Rajah or a Nawab During this stage he may walk along the streets continuously for hours without feeling fatigued His eyes may be glued on to the ground and he imagines he is looking for gold and treasures, instead he picks up rubbish and stones from the ground and other articles thinking them to be riches and articles of value These he carries on his person till he recovers from the effects of the dose and finds them to be useless During this time he is afraid of being robbed and fears everyone he meets The fear of excise authorities or the police are prominent among the hallucinations occurring among the poorer class of addicts in India The indication of the maximum amount of hilarity is marked by coldness of finger ends and dilation of the pupils

When chewing the betel leaf the addicts as a rule have a marked objection to talking As the leaf is chewed the mouth becomes full of saliva and there is a pleasant sensation as if the whole cavity is full of butter which spoken words might dissipate The addicts as a rule do not swallow the saliva but retain it in the mouth and give it time for absorption from the buccal cavity The reason for this appears to be that if some of the saliva containing cocaine gets into the œsophagus and the stomach it gives rise to a feeling of constriction and discomfort in the throat and the chest Besides this the drug is more quickly absorbed from the stomach and produces a more intense action which in the unimitiated may be so severe as to produce a mild collapse Ordinarily these symptoms consist of confinement, an hunger, feeling of severe oppression in the chest, dizziness and heaviness in the head They therefore do not swallow the saliva to escape the concentrated effects of the drug The addicts also firmly close their lips and avoid talking to friends, when they are chewing a 'pan' containing cocaine They are afraid that if they speak some of the drug may trickle down into the gullet and stomach or may flow out of the mouth The old and experienced addicts, however, do not hesitate to swallow the saliva to get stronger effects from the dose Those who are new to the

drug and are afraid of its strong effects expectorate the saliva after chewing the betel leaf

The stage of excitement after cocaine lasts from 45 minutes to an hour or an hour and a half There was a good deal of difference of opinion among the addicts regarding the duration of the stimulant stage undoubtedly due to the fact that most of the drug supplied nowadays is adulterated to a greater or lesser extent The addicts who have been taking cocaine for long periods definitely stated that the pure drug which they used to get in old days had a much stronger and lasting action than the adulterated commodity they are getting at the present time The effects, they are emphatic, certainly lasted 1 hour to 1½ hours They also said that in six months the pure alkaloid reduced the victim to a state of physical and mental wreck, he became pale and thin like a straw as many of them put it The intoxicating effect of the heavily adulterated drug obtained at the present time, according to the majority of them, did not last for more than a quarter of an hour

During the stage of excitement the addicts like the company of other addicts and they mutually persuade each other to indulge more and more in the drug leading to long continued cocaine debauches The advanced hours of the night do not induce them to retire to sleep as during cocaine intoxication sensation of sleep is entirely absent In this respect cocaine differs from other euphoric drugs such as opium, *cannabis indica* or alcohol which sooner or later produce drowsiness and sleep Many addicts related how sometimes for 2 or 3 nights when indulging in cocaine they did not close their eyes When, however, the debauch was finished and the intoxication passed off, they felt the effects of their vigil in the form of an acute feeling of physical and mental fatigue, headache and extreme misery, till they dropped down to sleep from sheer exhaustion If a fresh dose of the drug is taken, even in this stage, it has a wonderfully reviving effect the victim feeling more or less normal for the time being and capable of doing work, but the depression which follows is much worse It is for this reason that there is an irresistible desire to repeat the dose again and again The strong craving is to get over the loathsome depression When the drug is available such doses may be repeated many times and deaths have been recorded from cocaine poisoning on account of overdosage If the dose cannot be had the inebriate feels absolutely lifeless and dejected and prefers to be left alone He feels as if he is going to die During the stage of depression the patient experiences strange fear of happenings of unpleasant things and persuades himself to take a fresh dose The depression of spirits, however, seems to be more imaginary than real for there is no fall of temperature, no effect on the pulse, heart or respiration The tongue and lips become moist again, the perspiration on the forehead stops altogether During this period the victim avoids the company of his friends and associates and has a feeling of hiding himself away from the world He may lie down in a quiet dark corner refusing to speak or to face his friends During this stage also the addict may suffer from maniacal symptoms such as hallucination chiefly taking the form of persecution

by the police or excise authorities He imagines that the police are coming after him, any noise or sound of footsteps startles him A large number of addicts are subject to paræsthetic sensations such as formication and crawling of insects or snakes or lice under the skin Others get intense itching all over the body A number of cases of insanity have followed cocaine habit

While the individual is under the influence of cocaine there is complete loss of appetite, there is no desire for food and the inebriate will reject the daintiest dishes offered to him, often a very intense feeling of thirst appears The victims suffer from a very obstinate type of dyspepsia which is believed by him not to yield to any other treatment except a dose of cocaine On account of its stimulating effect on the ganglia of Auerbach's plexus a dose of the drug in many addicts produces rapid evacuation of the bowels immediately after it is taken This symptom was particularly noticeable in the Delhi addicts Soon after taking the dose they have to run to the latrine where they remain seated for a considerable time absorbed in their delusions Constipation, however, is a commoner symptom, it is the most distressing symptom occurring in cocaine addicts and was given by many as the reason for the repetition of the dose They said that the bowels were confined and there was a very unpleasant sensation of fullness in the abdomen, the feeling was described as if the inside was full of stones This was readily relieved when they took a dose of the alkaloid the effect being so strong that a rush had to be made to the latrine Some of the old-standing addicts were not much worried by constipation or other gastro-intestinal symptoms

Another distressing symptom occurring in cocaine addicts is insomnia Addicts keep wake all night desiring a fresh dose of the drug They simply cannot sleep try as they may Even the ordinary hypnotics appear to produce little effect in relieving this condition Most of the cocaine eaters complain of insomnia and it is very often for this complaint that they consult a medical man In a few of the addicts who took fairly large doses sleeplessness was not complained of Delusion and hallucinations greatly disturb the mental equilibrium of the cocaine eaters and gradually makes them most miserable The prolonged use of the alkaloid is also said to bring about deafness and confirmed cocaine addicts are said to be often slightly deaf We noticed this symptom in a number of our cases

Toxic effects—In case of stronger intoxication, be it through bigger doses or abnormal sensitiveness of the individual, hallucinatory symptoms quickly appear These hallucinations as already stated are of the nature of persecution such as fear of house-breakers, police and paræsthesia of the skin Sometimes especially after larger doses have been taken, there is a feeling of sickness, nausea, vomiting and cramps in the muscles If toxic doses of cocaine are taken the patient becomes semi-conscious, gets twitching of the muscles of the face and general tremors of the body followed by convulsions The body temperature shows a considerable tendency to rise Convulsions can be easily controlled by sedatives and, if severe, inhalations of chloroform must be given Very often

they pass off, the patient gaining consciousness but feeling utterly exhausted and miserable A dose of cocaine in this condition revives the victim Paralytic symptoms appear if very large doses are taken and are followed by coma and death from stoppage of respiration The toxic symptoms appear only after toxic doses or prolonged indulgence

Abstinence symptoms—The foremost of these is a strong craving the addicts show to repeat the dose After the effect of one dose is over the desire for the next dose we have pointed out is almost irresistible The one idea in the addict's head is to get another dose, and he will do anything to satisfy this craving There is such a strong desire in some individuals for the drug that the habitue will commit any kind of crime, or woman would even sell her honour to get the drug The victim feels restless, irritable, unable to concentrate and quarrelsome There is a great disinclination for mental and physical exertion, the addict feels dull, drowsy and lazy, in fact is in a condition of complete lethargy and inertia There are vague symptoms all over the body and the gastro-intestinal functions are depressed Sometimes the symptoms of constipation, cramps and sensation of formication, etc, are delayed, not appearing for 3 or 4 days after the debauch Rarely patients get so depressed that they feel they are going to die, they may become prostrated and collapsed Such symptoms are met with much less frequency in case of cocaine than with opium and morphine A large number, especially those taking large doses, lose all interest in their life and surroundings and develop suicidal and morbid tendencies All these symptoms entirely disappear for the time being, if a dose of the drug is taken

Time of taking the drug and dosage—Cocaine is generally taken by the habitues late in the evening or in the early hours of the night Persons engaged in dissipation, who are confirmed and inveterate eaters, take it during all hours of the day in betel leaf Many of the artisans and workmen we saw in *Delhi* do not take the drug except during the evenings when they have finished their day's work Others take it twice a day They generally take one or two doses of the drug, as much as they can afford, and then go back home to rest They said that the effect of one dose lasted them for 6 to 8 hours and if they could get it they were able to carry on their daily vocation without any difficulty Occasionally, however, these people go on taking the drug all night, especially when they are in the company of prostitutes and women of low morals

The daily dosage consumed shows wide variations The drug is very expensive and difficult to get, besides it is often very heavily adulterated The tendency for increasing the dose is irresistible and in a short space of a few weeks the dose may be increased to 20 or 30 grains Moneyed people as a rule take much larger doses than people with smaller means because they can afford to buy more of the drug Most of our addicts took doses ranging from ½ to 15 grains, the latter being the maximum limit among the ordinary

murmurs may be heard Besides this the alkaloid especially in large doses has a toxic action on the myocardium and in old-standing addicts and those taking large doses the pulse is weak and blood-pressure low Even after moderate doses there is stimulation of the sympathetic and depression of the vagus but after the stimulation there is generally a paralytic condition of the sympathetic system

As regards the effect of the drug on the hæmopoietic system there is no doubt that the drug in all probability has a direct action on the blood forming organs especially when taken in large doses It also produces an indirect action by producing intestinal stasis and toxæmia Malnutrition is brought about from want of food from loss of appetite, distaste for food and digestive disturbances which are set up by the alkaloid This has a deleterious effect on the hæmopoietic system in the same way as it has on the other systems

Respiratory system—Soon after taking the dose the respiration is quickened When larger doses are taken there is marked quickening at first, the respiration becoming deeper and more frequent This effect is undoubtedly produced by the stimulation of the respiratory centre With toxic doses there is slowing and the respiration may assume the Cheyne-Stokes' type

Genito-urinary system—The amount of urine in cocaine eaters is said to be diminished, but we have not been able to carry out any observations on that point Probably this is due to smaller intake of both food and drink We have examined the urine of a number of addicts in our series but found no abnormal constituents, the quantity of the alkaloid, if it is excreted by the kidneys, is probably too small to be detected by the ordinary alkaloid precipitating reagents None of the addicts complained of passing abnormally small quantities of urine, but tenesmus of bladder was observed especially after large doses Women taking cocaine suffer from backache, leucorrhœa and often from amenorrhœa and dysmenorrhœa

Nervous system and special senses—As a result of cocaine toxæmia the central nervous system is the greatest sufferer, the highly developed nerve cells of the grey matter suffering most of all These cells are at first violently stimulated and the stimulation is followed by a reactionary depression The symptoms detailed under mental effects such as delusion, hallucination, impairment of character and low state of mental efficiency and sometimes a sort of chronic insanity called cocaine paronoia can all be explained

On the higher psychic areas the drug has at first a marked stimulant action followed by a strong paralytic action The habit almost invariably leads to mental and moral deterioration After it has persisted for some time there is great impairment of intelligence and loss of memory The addicts become dull and stupid and acquire lazy and dirty habits There is great weakness of will power and inability on the part of the individual to concentrate over subjects which require deep thought A large majority of the addicts suffer from hallucinations of sight and hearing Delusions of the nature of persecution are very common and were met with in at least 30 per cent of

our series Cocaine paranoia is of frequent occurrence, the addict suffering from hallucinations of sight and hearing, delusion of the nature of persecution, which make him so timid and foolish that he startles at the slightest noise Prolonged use and excessive indulgence lead to great mental impairment and a number of people drifts towards insanity In our series there were a few cases of epilepsy, the fits starting after the commencement of the drug habit Some suffered from suicidal and homicidal impulses, the former being more common All these effects are no doubt brought about by the persistent use of the drug bringing about structural changes in the nervous tissue The most distressing of all symptoms is insomnia which is of a very obstinate nature

There may be widening of the palpebral fissure and the cornea may have a glassy appearance, the eyeballs also protrude owing to sympathetic irritation In 2 of our cases at least there was impairment of vision and hearing is generally impaired The deafness occurring among the addicts is probably due to the paralysing effects of the alkaloid on the auditory nerve endings The superficial and deep reflexes are not affected There is paralysis of sensory organ after local application The skin becomes pale, there is loss of subcutaneous fat, and in some cases a peculiar rash is observed Paræsthesias in form of intense itching of the skin followed by a sense of creeping of worms and insects under the skin or embedding of sand and pebbles under it are frequently met with (in 30 to 40 per cent of addicts in our series) The paræsthesias appear at times and disappear

Sexual effects—Cocaine is the most important addiction with regard to its effects on the sexual life As mentioned before in this country it is closely associated with sexual vice and prostitution A fairly large number of addicts in our series gave the history that they started the habit in company of some female paramour or a prostitute Cocaine it has been said is popularly supposed to possess aphrodisiac properties and quite a large number of our series of cases started the drug for its alleged stimulating effects on the sexual faculties We carefully questioned all our patients in this respect but none of them could say that the drug possessed any marked stimulant effects on the sexual appetite During general stimulation of the higher parts of the brain which follows this drug, there is undoubtedly sharpening of all the senses and faculties and this may produce a semblance of sexual stimulation Even in this state the alkaloid has no specific exciting effect on the sex organs either in the male or in the female It has been stated that the time up to ejaculation is highly extended in man Most of our addicts informed us that the sexual act is not in any way strengthened or prolonged, as a matter of fact they all held that it was distinctly weakened During cocaine intoxication the addicts said it was impossible to perform the sexual act It was during the depression period that according to some the act was considerably strengthened and ejaculation time was markedly extended

As regards the effect of the drug on the female sex, our experience in this respect has been very limited being confined only to a few prostitutes In his

able memoir on cocaine addiction Professor Hans W Maier (1926) stated that owing to stimulation of the psychic areas bodily erotic attractions increase in those that are inclined that way Cocaine addiction in such individuals is generally of sexual origin, and they show all possible perverse symptoms and loss of all moral control Homo-sexuality occurs among those indulging in the drug and we found this to be the case in some parts of the United Provinces and other places among those indulging in the drug The sexual depravity is undoubtedly due to loss of control of the higher centres which is present under normal conditions and is absent in cocaine eaters Hetero-sexuality during cocaine intoxication may change to homo-sexuality Many of our addicts definitely told us that the sexual desire certainly increased among women addicted to the drug

A large number of the inebriates stated that cocaine has a distinct depressant action on sexual functions in all stages A few said that in the beginning the sexual act was somewhat prolonged but a distinct weakening effect was produced later on in the course of addiction Others stated that they felt no sexual desire while under its influence, the desire only returned after its effect had passed off when he or she felt voluptuous feelings Still others definitely stated that they did not get erections while under the effect of cocaine then only desire at that time being for a fresh dose of cocaine These people felt sexually fit only when the intoxication produced by the alkaloid had entirely ceased

It would be interesting to note here that not a few of the addicts, especially those who had taken the drug for a long time, complained of sexual neurasthenia and complete loss of sexual desire and disinclination for the company of the opposite sex In women it leads to amenorrhœa, dysmenorrhœa, leucorrhœa, backache, lack of sexual desire and sterility

Social and moral effects—Cocaine eaters are looked down upon by the general public in India The addict is contemptuously called 'Cocaine Baz' (one indulging in cocaine) and is intensely disliked by those who know him Almost all addicts we met were persons of low morals, they become habitual liars and pilferers No one would trust them in business matters and their evidence in court is not relied upon Their company is considered to be objectionable by their neighbours and relations They lose all sense of self-respect and would resort to anything, however, despicable or criminal to secure their daily dose when that is not forthcoming by fair means Several of the victims to the habit informed us that they simply took the dose from others by force if they did not have it themselves It has been stated that the reason why cocaine among all other narcotic drugs deserves the fullest attention is firstly because of the easy way of its application (i e, by eating in 'pan'), secondly, for the peculiar aspect of the habit which is not indulged in isolation like some of the other narcotics but spreads like a contagious disease and lastly for its disintegrating influence on the character of the victim, paving the way to prostitution and crime It has become known to police departments of the

western countries that a criminal devoted to cocaine is far more ready to use firearms than an ordinary crook, who does not usually possess them We have not studied the cocaine habit in India sufficiently to be able to say whether what is true in this respect of western countries is also true of India So far as our experience in this country is concerned the cocainist is more dangerous to himself than to other members of the society from a criminal point of view Many cocainists of the artisan class we examined carried out their daily vocations and only indulged in the drug in the evening for an hour or two and then returned to their homes or spent the night in the company of some women They took the drug just to experience the exquisite feeling, short though it is, given by the drug When asked to describe what sensation they felt they informed the authors that they could not describe them in words, in the same way as they could not describe the feeling of pleasure obtained during the sexual act They all said that the sensation was so wonderful and fascinating that they were prepared to face all consequences to get it even for a short space of time

Many of the addicts said that they took the drug because of the severe constipation from which they suffered and because of the most distressing feeling in the abdomen they experienced neither of which could be relieved except by a dose of cocaine They all loathed the habit and wished to get rid of it They begged us to find something which could help them to get over the craving They all remembered their first introduction to the drug and cursed the person who was responsible for giving them the habit

Strength of the habit—Cocaine habit once formed is very difficult to give up, although it is believed by some to be not more difficult to abandon than the opium habit Prolonged use leads to mental deterioration and weakness of will power, so that the individual seldom has the strength to resolve to give it up The withdrawal symptoms already described specially insomnia, cramps in the muscles, constipation, disturbance in the abdomen, etc , make it all the more difficult for the drug to be abandoned With proper guidance, complete removal from the environments, improving the physical condition and vitality of the patient may help him to resist it

Diagnosis—In western countries a cocaine addict can be easily recognized from his general appearance and from scar marks of injection Those who take it in form of snuff get '*rhinitis chronica*' which may go on to ulceration of the nasal septum and its perforation The presence of these along with other symptoms gives the patient away In India it is not difficult to diagnose a case of cocaine addiction after one has seen a few cases The following are the chief points which help in diagnosing a case of cocaine addiction —

(1) Appearance of the addict is typical He is generally young, emaciated with sallow complexion, sunken eyes, wrinkled face and dilated pupils

(2) The addicts have a typical dark chocolate coloured deposit on the teeth and the tongue This deposit is more marked on the incisors and the canine particularly on their posterior aspect

(3) Such symptoms as hallucination, delusions of persecutory nature, moral and mental degeneration give the cocainist away

(4) Paræsthesias such as creeping sensation like that of lice, worms, snakes under the skin (cocaine bugs) are pathognomonic of this addiction

Treatment—The treatment is general The most important points to be remembered are —

(1) Removal of the addict from the environments in which he learnt the habit and from the associates in whose company he indulges in the drug, preferably to a place where he cannot get the drug This is very important as we have known many instances where the individuals from Delhi or Saharanpur went for a few months to the Punjab where they were unable to get the drug and where there were no associates and they were able to conquer the craving for months at a time When, however, they returned to their old surroundings (where they were able to obtain a supply of the drug) they again succumbed to the temptation Similar facts have been observed in cases of addicts who have been imprisoned They are able to give up the habit in jails and go for years together without it, but immediately take it up again after being discharged from the jail

(2) Psychotherapy, mental training and impressing upon him the gravity of continuance of the (drug) cocaine habit are very important The patient should be encouraged to have a firm mind and to give up the habit

(3) The drug must be withdrawn all at once

(4) The rest of the treatment is simple and symptomatic

(*a*) Cramps and insomnia should be treated by simple means such as massage, exercises, hydrotherapy (and warm baths) If there is need for a sedative give bromides, chloral paraldehyde should be used with caution while hypnotics such as opium and morphia are strongly contra-indicated General tonics like iron and strychnine should be given Quinine, strychnine, iron and arsenic in pill form are very useful Constipation which is a very troublesome symptom should be treated by simple means such as warm soap enema or saline purgatives Drastic purgatives are not recommended

Diet should be simple, easily digestible and nutritious such as milk, soups, vegetables (minced and well cooked), and fruit juices Feranacious articles, underground vegetables and diet rich in fats should as a rule be avoided in the early stages of treatment

Summary and conclusions

(1) Cocaine habit is of comparatively recent origin in India The earliest records of its use are not more than 40 to 45 years old

(2) *Erythroxylon coca* has never been collected in India on a large scale, so far as is known no other varieties of Erythroxylon which bear cocaine and grow in India in a state of nature are cultivated There is no ground whatever for the belief that cocaine is secretly manufactured in India

(3) The leaves of *E coca*, which have been largely indulged in by the inhabitants of South America for several hundred years, have never been used in India for their euphoric effects It is the alkaloid cocaine which was and is still used

(4) The habit was prevalent in many towns in India in the beginning of this century and the evil effects produced by it came to the notice of the medical profession and the authorities about the same time Restrictions were at once placed on the import, sale, possession, transport and use of the alkaloid and the preparations in which it was contained

(5) In spite of this the habit spread from Calcutta to large towns along the two main railway routes through the United Provinces into the Punjab and the North-West Frontier Province From Bombay it spread to some of the large towns in that presidency

(6) The drug is smuggled into India in large quantities through sea ports particularly Calcutta and is distributed to different parts of the country by the smuggling organizations For some time past the Far East has driven the European and American manufactured product out of the market, Japan being the chief source now The drug is illicitly brought by crews of various line of mercantile marine running between Japan and Calcutta

(7) It is not possible with any degree of accuracy to gauge the present extent of cocaine habit in India but a careful survey of the problem shows that the habit is chiefly prevalent among the betel leaf chewing population of north-western portions of India (including Bengal, Bihar, United Provinces and the Punjab) The general distribution of the habit is given in the map It is estimated that about a half to one million cocaine eaters belonging to different strata of society exist in India The habit is not only present among the well-to-do population but a large number of the artisan class in large towns are also victims of the habit

(8) Cocaine sold in India is very heavily adulterated, the chief adulterants used being phenozone, phenacetine, caffeine citrate, acetyl salicylate and potassium nitrate

(9) Two hundred cocaine addicts have been carefully studied and analytical data regarding the causes leading to the habit, the duration of addiction, occupation of the addict, dosage, sex incidence, its effects on sexual functions and fecundity, association of cocaine habit with other drug habit, etc, have been discussed

(10) Of the main causes leading to addiction, association with other addicts is an important factor accounting for as many as 66 0 per cent of cases in this series, luxury and pleasure come next accounting for 21 per cent, fatigues worry and strain for 6 5 per cent, curiosity and fancy for 4 5 per cent and disease for 2 per cent The habit is chiefly confined to individuals whose psychic condition is in an unstable state of equilibrium

(11) The most common method of taking cocaine in India is by putting it in a 'pan' or betel leaf The drug is either mixed with the spices which

are usually put in the leaf, or some of the addicts place the alkaloid on the dorsum of the tongue and then chew the leaf afterwards Addicts of long standing generally put cocaine on the tongue and follow it up by a little lime and catechu, dispensing with the betel leaf This is said to enhance the action of the drug The method of taking the drug by injection or taking it in the form of snuff is unknown in this country

(12) A complete account of symptoms produced by cocaine eating as observed from a study of a series of 200 cases have been described in detail and the deleterious effects of the habit on the physical, mental and moral condition of the addict have been discussed

(13) Diagnosis of cocaine habit and its treatment have been described The appearance of the addicts is typical They are emaciated individuals with a sallow complexion, sunken eyes, wrinkled face and dilated pupils They have a dark chocolate coloured deposit on the teeth and the tongue which is considered to be pathognomic (*see* Plates LIII to LV)

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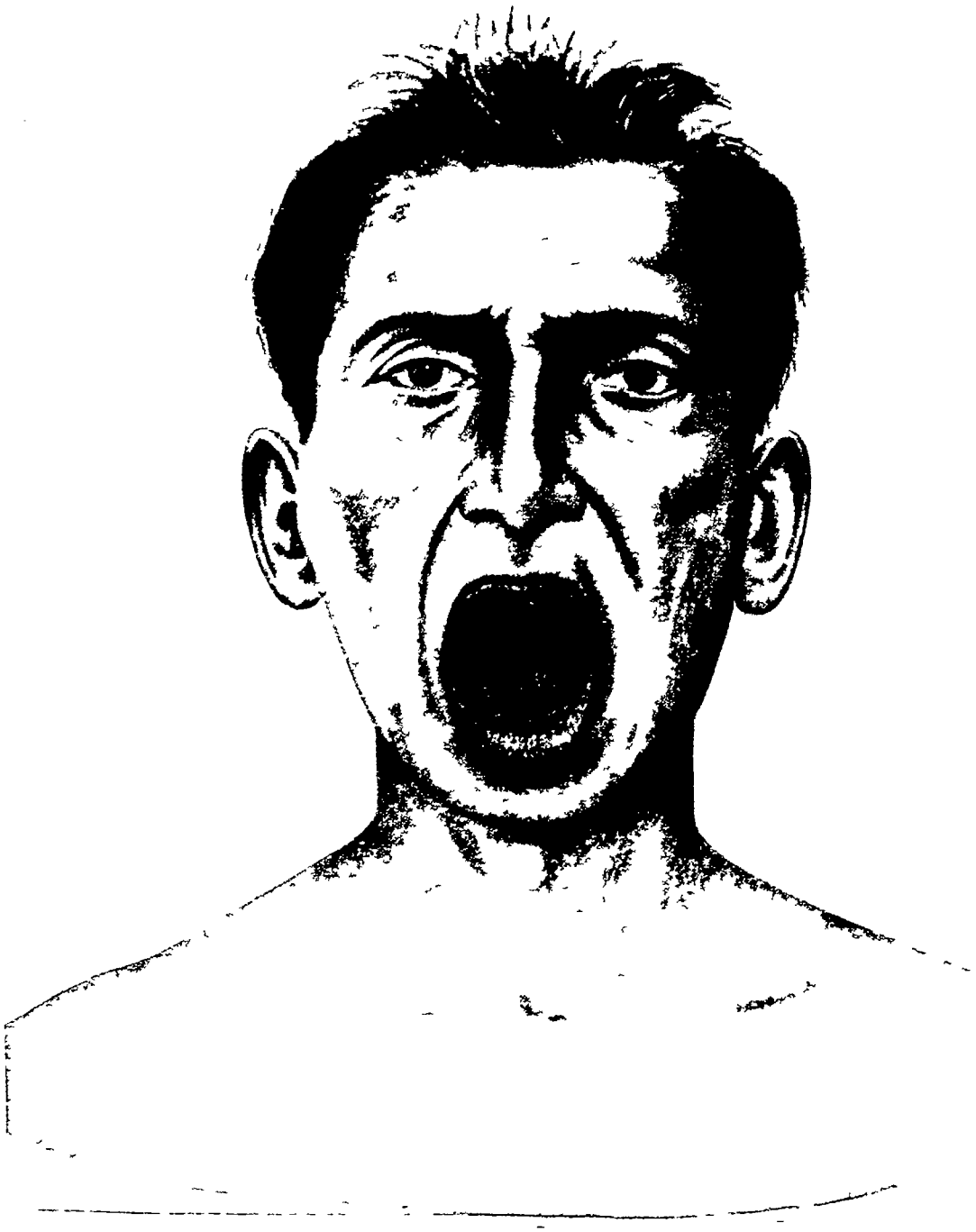
PLATE LIII

Shows three cocaine addicts of longstanding duration from the artisan class



Shows the typical discolouration of the tongue and teeth in cocaine addicts of longstanding duration Note the cracked appearance of the lips

NOTE ON THE FEEDING HABITS OF *PHLEBOTOMUS MINUTUS*

BY

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[Received for publication, October 14, 1930.]

IN the July 1930 number of the *Indian Journal of Medical Research* there is a paper by Lloyd, R. B., and Napier, L. E., on 'The Blood-Meal of Sandflies Investigated by Means of Precipitin Antisera.' This paper contains statements so contrary to previous ideas with regard to the feeding habits of the *minutus* group of the genus *Phlebotomus* that some explanation seems to be called for.

The following statements are made in the authors' summary:

1. '*P. minutus* also usually feeds on the blood of cattle or man, but also quite frequently on the blood of other species.'

2. *P. minutus* is a more persistent human-blood feeder than *P. argentipes*.'

Howlett (1913) pointed out that *P. minutus* normally fed on reptilian blood (gecko). My own experience, and that of my colleagues on the Kala-azar Commission with whom I have discussed the point, shows that this is correct, and that *P. minutus* (*minutus* group) never feeds on man in Assam. Although, in the past, we have repeatedly tried in vain to feed flies of the *minutus* group on man without success, I arranged for a special experiment to be carried out, and the results of this are recorded below in tabular form. The plan followed was to give numbers of flies of the *minutus* group two opportunities, totalling

about 3 hours, to feed on man, and then to give them one opportunity to feed on geckos The results were as follows —

Date	Number of flies of *minutus* group used	Number fed on man (exposure 3 hours)	Number fed on gecko
9-8-30	9	Nil	9
10-8-30	35	„	31
11-8-30	55	„	20 (some died)
12-8-30	37	„	25
13-8-30	41	„	29
14-8-30	31	„	12 (many died)
15-8-30	22	„	15
16-8-30	26	„	14
20-8-30	39	„	12
21-8-30	44	„	30
Totals	339	Nil	197

These figures appear to me conclusive and would point to certain fallacies in the results recorded in the paper in question

(*a*) The identification of the flies may have been at fault

(*b*) The technique employed in the precipitin tests may be unreliable

In the latter case this would also invalidate the results obtained with other species of *Phlebotomus* described in the same paper

A further point which may be raised is that the authors do not state whether they have ever seen *P minutus* actually feeding on man In flies of the *minutus* group which we have caught in Nature in Assam, the blood, when recognizable, has always been composed of nucleated red cells

As regards the question of identification, the authors, in the paper referred to above, speak, throughout, of *P minutus* It is possible, however, that they may have been dealing with different members of what is often loosely referred to as the '*minutus* group' of the genus *Phlebotomus* It is possible that some members of the group may be mammalian feeders although there is no definite evidence on this point The question is of some importance, from the greater biological point of view if it is true that at least some members of the *minutus* group in Calcutta feed on man, and from the lesser medico-legal point of view if they do not, since, in the latter case, the precipitin test in the case of insects would appear to be fallacious

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ON A NEW SPECIES OF CULICOIDES (*CULICOIDES CLAVIPALPIS* SP NOV), WITH NOTES ON THE MORPHOLOGY OF THE MOUTH-PARTS AND MALE TERMINALIA OF AN INDIAN CULICOIDES

BY

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Ancillary Inquiry into the Transmission of Kala-azar, Calcutta

[Received for publication, October 17, 1930]

CONTENTS

1 Introduction
2 Description of female *Culicoides clavipalpis* sp nov
3 Morphology of the mouth-parts of a Culicoides
4 Morphology of the male terminalia (Hypopygium) of a Culicoides
5 A tentative key to some of the Indian species of Culicoides
6 Summary and conclusion

1 INTRODUCTION

IN carrying out investigations on the transmission of diseases, especially, those caused by insect vectors, the value of a thorough entomological survey carried over the endemic areas and their adjacent regions cannot be gainsaid During the early part of 1929, a systematic survey of blood-sucking insects of Calcutta and its environs was started Special attention was paid to the genus Culicoides and a number of engorged females from each day's catch was systematically identified in the laboratory and submitted to another department for accurate determination of the nature of their blood-meals Until now most of the engorged females were identified as *Culicoides peregrinus*

Kieff,* and *C ovystoma*, Kieft, (?) *maculithorax*, Willst, while a couple collected from cowsheds in the outskirts of Calcutta proved to be new to Science It is proposed to describe in the following pages the new species Short notes on the morphology of the mouth-parts and male terminalia of a Culicoides are also being appended All the drawings were made by me with the camera lucida (eyepiece type)

I would like to extend herein my indebtedness to my chief, Dr L E Napier, M R C S (Eng), L R C P (Lond), for the generous and kind way in which he went through the paper

2 Description of *Culicoides clavipalpis* sp nov, ♀

Coloration

Head yellowish brown, except the eyes, palpi sparingly clothed with long brownish hairs, clypeus (Plate LVI, fig 2 *cl*) light brown decked with few fine long brownish hairs, antennæ brownish yellow, mouth-parts (Plate LVI, fig 2 *pp*) light yellow, eyes (Plate LVI, fig 2 *ey*) black with a triangular light coloured area in between, on the occipital region surface of head covered by fine brownish chætæ, occiput (Plate LVI, fig 2 *oc*) sparsely clothed with long brownish hairs, dorsum of thorax yellowish brown sparsely clothed with medium brownish hairs, mesonotum yellow brown, mesoscutellum of a deeper colour sparsely clothed with long brownish hairs, mesopostnotum of a slightly lighter colour, abdomen brown dorsally, somewhat lighter ventrally, halteres yellowish white, legs brownish yellow, tarsi pale

Wing coloration (Plate LVI, fig 1) characteristic and consists of paler areas on greyish background Near the centre of the upper margin where the 1st and 3rd longitudinal veins terminate in the costa after forming the radial cells, an irregularly forked rather broad clear area is present the upper branch of which reaches the costa while its lower branch terminates about the middle of the upper branch of the 4th vein, one clear roundish area is present near the region of the radio-median cross-vein, another is situated dorsal to the terminal end of the upper branch of the 4th vein, between the branches of the 4th vein towards the apical half are two clear roundish areas, a clear rather

* As early as July 1913, Dr N Annandale records from Balugaon in Orissa that *Culicoides peregrinus* Kieff, bites human beings His own version runs as follows 'The type specimens of *C peregrinus* were taken at Puri on the coast of Orissa in March I recently (July 1913) found the species very abundant in a bungalow near Balugaon in the same district One individual was killed in the act of biting my wrist and I had reason to think that many others were attacking my ankles The irritation was considerable but not lasting and very little swelling followed the bite Both sexes swarmed at night in the corners of rooms, particularly in the neighbourhood of a lighted lamp females were much commoner than males' Mr H Stevens who collected specimens of *C himalayæ* Kieff, at Kalimpong on the Nepal-Sikkim Frontier at an altitude of about 9,000 feet, refers to them as 'blood-sucking flies of particularly venomous nature' Recently Smith (1929) records two new species of Culicoides (*C ravipalpis* and *C actoni*) from Assam which bite man

irregular area occupies a position just below the origin of the lower branch of the 4th vein while another clear spot rather indistinct and of irregular shape is situated a little beyond this towards the base of the wing, below the lower branch of the 4th vein and dorsal to the 5th vein are two roundish clear areas, a clear oval area exists between the branches of the 5th vein while another roundish clear area is present a little beyond the region where the 5th vein forks Altogether there are 11 pallid areas distributed throughout the wing surface The area occupied by the radical cells is darker than the ground colour, a small strip dorsal to the unbranched portion of 4th vein slightly darker, a small strip below the termination of costa light brown The wing membrane is uniformly covered with microtrichia* the colour of which varies according to the respective areas they occupy Macrotrichia are distributed sparingly throughout the apical half of the wing surface but not on the pallid areas A few macrotrichia are present in the courses of the branches of the 4th vein near their terminations

Structure

Head (Plate LVI, fig 2) eyes large contiguous dorsally and leaving a triangular area (*ti a*) in between, the area occupied by the eyes covering nearly $\frac{3}{4}$th of the dorsum of the head The head, broader laterally, oval in shape At its junction with the neck the head bears marginally a small protuberance bearing dorsally an oval light coloured area, maxillary palpi (*mx p*) with the second joint (Carter's third) considerably swollen and expanded on both the inner and outer sides, the two apical joints sub-cylindrical, the three distal joints related in length in the proportion as 24 7 8 The swollen joint bears a large sensory pit (*s pt*) oval in outline Antennæ (Plate LVI, fig 3) with segments 2–9 subglobular to cylindrical apically, 8–9 nearly equal, related in length and breadth as 8 5 respectively, verticels 6 in number arranged in a single whorl at a distance of about 1/3rd from the base of a joint, each more than twice as long as the segment, sensory hairs about 3–4 in number on each segment Segments 10–14 and 2–9 related in length in the proportion as 16 13, segments 10–13 nearly of equal length, 13–14 related in length in the proportion of 3 4 Mouth-parts (Plate LVI, fig 2 *pr*), *vide* a detail description of mouth-parts given in Section 3 *Legs* (Plate LVI, fig 4 *A B*) having general structure of Pangonine type in that the hind legs bear tibial spurs, fore leg tibiæ (Plate LVI, fig 4 *A*), apically armed with a few spines, the tarsal joints related in length in the proportion of 15 6 5 3 4, the mid leg of nothing particular of note, in the hind leg (Plate LVI, fig 4, *B*) tibiæ bear apically a few long brownish spines in addition to the rather dark paired tibial spurs (*sp*) The tarsal joints related in length in the proportion of 34 17 11 8 9 *Wing venation*, 1st and 3rd veins (Plate LVI, fig 5 *1, 3*) when present rather faint, hence the radial cells difficult to distinguish, sub-equal, very narrow and their line of demarcation not distinct (Plate LVI, fig 5 *r* c_1, *r* c_2), radio median

* Microtrichia have no basal articulations

3 Structure of the male terminalia (Hypopygium) of a Culicoides has been dealt with in detail and a comparative study of the structures has been made An attempt has been made to homologize the appendages of the ♂ hypopygium It is concluded that as in other groups of insects the male terminalia are likely to play an important part in the correct identification of the Indian forms

4 A tentative key to the Indian species of Culicoides mainly based on the structure of the wing, spermathecæ, and male hypopygium has been appended

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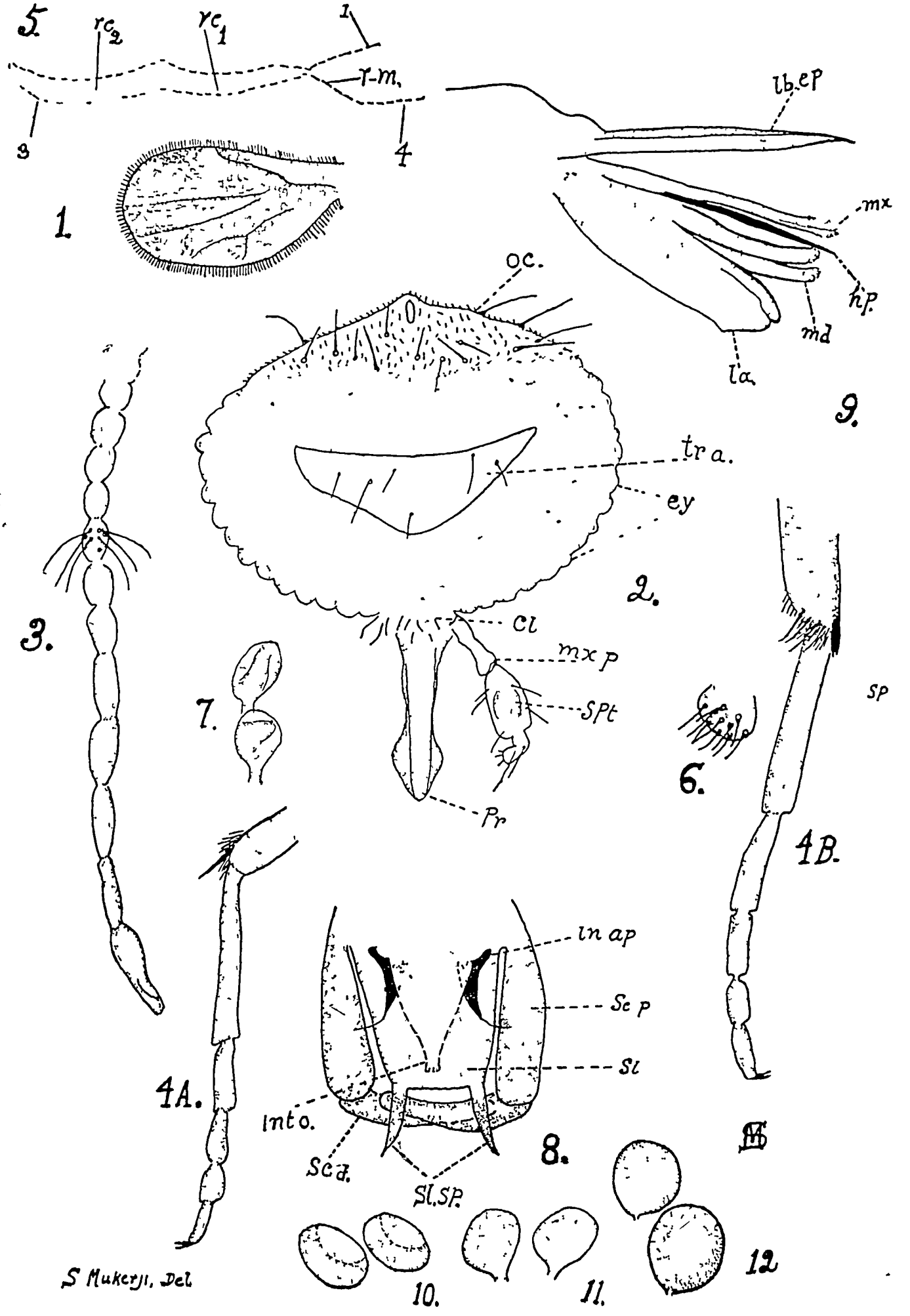
PLATE LVI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Explanation of Plate LVI

Fig 1 Left wing of *Culicoides clavipalpis* sp nov ♀ × 60

„ 2 Head of *Culicoides clavipalpis* sp nov ♀ (viewed from dorsal aspect) × 300

„ 3 Some of the antennal joints of *Culicoides clavipalpis* sp nov ♀ × 300

„ 4A Portion of fore leg of *Culicoides clavipalpis* sp nov ♀ × 300

„ 4B Portion of hind leg of *Culicoides clavipalpis* sp nov ♀ × 300

„ 5 Radial cells and radio-median cross-vein of wing of *Culicoides clavipalpis* sp nov ♀ × 300

„ 6 Eighth abdominal sternite of *Culicoides clavipalpis* sp nov ♀ × 300

„ 7 Spermathecæ of *Culicoides clavipalpis* sp nov ♀ × 300

„ 8 Male terminalia (Hypopygium) of *Culicoides* sp × 300

„ 9 Mouth parts of *Culicoides* sp × 300

„ 10 Spermathecæ of *Culicoides peregrinus* Kieff × 300

„ 11 Spermathecæ of *Culicoides oxystoma* Kieff (?) *maculithorax* Willist × 300

„ 12 Spermathecæ of *Culicoides himalayæ* Kieff × 300

Key to letterings in Plate LVI

cl	Clypeus
ey	Eyes (marginal ones shown)
hp	Hypopharynx
in ap	Intermediate appendage
int o	Intromittant organ
la	Labium
lb ep	Labrum epipharynx
md	Mandible
mx	Maxillæ
mx p	Maxillary palp
oc	Occiput
pr	Proboscis
rc_1, rc_2	Radial cells of wings
r-m	Radio-median cross-vein
sc d	Distal segment of superior clasper
sc p	Proximal segment of superior clasper
sl	Subgenital lamella
sl sp	Spines of subgenital lamella
sp	Tibial spurs of hind legs
s pt	Sensory pit of maxillary palp
tr a	Triangular area between the eyes
1, 3, 4, etc	The longitudinal veins

The following has been received from the War Office, London, dated the 5th September, 1930 —[ED]

NORTH PERSIAN FORCES MEMORIAL MEDAL

Captain H W MULLIGAN, M B , Indian Medical Service, has been awarded the North Persian Forces Memorial Medal for the year 1929 for his paper 'Studies on the Reticulo-Endothelial System, with Special Reference to Malaria,' published in *The Indian Journal of Medical Research*, Vol XVI, No 4, April 1929, pp 1099–1119

This Medal is awarded annually for the best paper on Tropical Medicine or Hygiene published in any Journal during the preceding twelve months by a Medical Officer, of under twelve years' service, of the Royal Navy, Royal Army Medical Corps, Royal Air Force, Indian Medical Service, or of the Colonial Medical Service, provided the Memorial Committee consider that any of the papers published has attained a standard of merit justifying an award

THE DISTRIBUTION AND CAUSE OF ENDEMIC GOITRE IN THE UNITED PROVINCES

BY

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INCORPORATING WORK BY

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[Received for publication, November 24, 1930]

IN certain well-defined areas of the United Provinces endemic goitre is exceedingly common. When the distribution of these areas is more closely examined, it is found that the goitre areas are associated with definite types of water-supply, and often with definite types of soil which are recognized as the cause of the goitres by the population concerned.

What then is the condition of these people with endemic goitre? How close is the connection between water-supply and endemic goitre? What is the apparent cause of endemic goitre in these provinces? Is this cause in keeping with the present view that endemic goitre arises from iodine deficiency?

For some five years past the Department of Pathology of King George's Medical College has been collecting data on these points. The *Government Census Reports* for 1881, 1891, 1901, 1911 and 1921 and the *Imperial Gazetteers for the United Provinces for India*, and indeed for each province in India in regard to goitre and its associated diseases have been examined and have furnished much valuable information. Successive research scholars have proceeded to the endemic districts and have collected with considerable industry much useful material which I freely incorporate here. Dr B B Bhatia, M.D., M.R.C.P., has been mainly responsible for the field work in Gorakhpur district, Dr K C Rai, M.B., B.S., for that in Gonda and Gorakhpur and Dr R S Lal,

MB, BS, for that in Basti, Bahraich and the Himalayas The whole area to be covered was so extensive that only small samples of each area could be investigated and a bird's-eye view of the goitre distribution in the several districts approximated

Further information was furnished by landlords and sugar planters of the affected areas, by Deputy Commissioners, Civil Surgeons and other medical personnel including members of the Public Health Department whose willing help is gratefully acknowledged Such information has been sifted and incorporated in the various sections Laboratory experiments were also undertaken, but had to be abandoned on account of the pressure of time for adequate supervision

The information thus obtained for the United Provinces is recorded in the following sections —

I Clinical observations on endemic goitre and on sub-thyroidism in endemic areas

II Distribution of deaf-mutism in the United Provinces

III Distribution of endemic goitre in the United Provinces and its relation to special drinking waters and soils of high calcium-content

IV Distribution of deaf-mutism and of endemic goitre in India

I Clinical observations on endemic goitre and on sub-thyroidism in endemic areas

An endemic goitre may be considered from three aspects (*a*) the neck swelling produced, (*b*) associated ætiological factors, and (*c*) the endocrine disturbance found in the endemic area

(*a*) *The neck swelling*

If an adolescent about puberty (i e, a young healthy wife) be brought from non-goitrous locality and exposed to the goitrous-producing influences of an endemic area, a swelling of the thyroid gland develops in some three or four months to a size varying from that of a full round neck up to about the size of a hen's egg This early goitre is soft in consistence and usually diffuse in distribution, though sometimes one lobe (more often the right) may be more prominent Should the person now leave the goitre area, this soft uniform swelling will probably spontaneously disappear within a few weeks Should the person return to the area, the goitre reappears, to again disappear if the area is again left If, however, the person continues to reside in the endemic area, the goitre usually gradually enlarges up to 30 or 40 years of age to a degree varying with the degree of endemicity of the disease and with the age of the individual At any time it may become stationary in size before finally somewhat slightly diminishing with the onset of fibrotic changes A common stationary size is that of a cricket ball At times, however, the goitre enlarges even to the size of a football, but this is rare, except in the areas of greatest

endemicity In old goitres the surface becomes markedly nodular and distorted by contraction of fibrous tissue and by the development of cystic or parenchymatous 'adenomata' Occasionally, practically the whole goitrous mass may be transformed into a hard fibrous lump, and various degrees of myxœdema ensue When such changes have arisen no medicinal treatment or change of residence to a non-goitrous area will effect removal of the tumour, though its size may become somewhat reduced Pressure symptoms on the various important neck structures may arise but are uncommon considering the degrees of enlargement attained

(*b*) *Ætiological factors*

(1) *Age*—In the United Provinces endemic areas, so long as an infant is suckling its mother's milk, i e, not infrequently up to 2 or 3 years of age, goitres are excessively rare In the Gonda endemic area 210 infants under one year were examined without finding a single goitre Between 3 and 5 years the rate is about 5 per cent, the goitres often first appearing at this age some 2 or 3 years after drinking goitre-water Of 2,070 children between 6 and 14 years 56 per cent had goitre Adolescents whether entering a goitrous locality from outside or born within it are always more susceptible than adults hence the incidence rate amongst them is at its height Adults coming to a goitrous area from outside not infrequently escape the deformity altogether, for the older the individual the greater are the goitrous influences required to produce a goitre and even if it does arise the smaller will that goitre be In the severest endemic areas the goitre rate works out in the following approximate proportion One to 2 years, 0 per cent, 2–5 years, 5 per cent, 6–12 years, 30 per cent, 12–15 years, 60 per cent, 15–40 years, 40 per cent, and over 40 years, 30 per cent

(2) *Education, poverty*—Very few well-to-do people acquire goitre Educated persons (zemindars, sub-judges, lower primary school teachers of whom there are about 60 in Gonda, merchants, station masters and assistant traffic managers, clerks and shop-keepers) frequently completely escape even in the worst endemic areas But there is no absolute immunity Education has taught them how to avoid goitre It is the poor uneducated ryot, labouring on and cultivating the soil, far removed from a non-goitrous water-supply who develop goitre

(3) *Sex*—If goitres are enumerated in any area there is an apparent greater ratio amongst males, but semi-purdah amongst the women greatly increases the difficulty of their enumeration Probably the sex distribution is approximately the same, or indeed may be even greater amongst females for it is recognized that goitres are especially apt to appear when the thyroid undergoes its cyclic physiological slight increase in size, i e, at puberty, during pregnancy and even during menstruation In certain villages, females without goitres are rare, and the swelling is indeed sometimes regarded as a desirable ornament amongst them

(4) *Family, community and race*—Goitre certainly runs in families because each member of the family living in an endemic area is exposed to the same goitrous influences Hence a child of goitrous parents is more likely to develop a goitre than a child of non-goitrous parents But inheritance does not play a part or at least a marked part in the production of goitre, for parents with goitres who remove to a non-goitrous locality do not seem to breed children with goitre Inheritance, however, is marked in sub-thyroidism and cretinism There is no apparent communal or racial proclivity apart from the influence of education and poverty

(5) *Species*—Goats, jackals and dogs are not infrequently affected I saw a dog with goitre in the Padrauna endemic area and placed it in the King George's Medical College Pathology Museum Birds are also said to develop goitre under natural conditions

(6) *Season*—New goitres especially arise, old goitres enlarge and all goitres most rapidly develop during and after the rains, i e, during October, November and December For it is, during the monsoon in the plain areas, that the rivers overflow their banks and alluvium with the goitre-producing substance in it is spread over the soil and readily reaches the shallow wells Attendance at out-patients at the various district dispensaries for goitre increases largely during the months following the rains (Chart 1)

(7) *Diet*—As the individuals affected are the poorest field labourers and cultivators who often cannot even afford a covering for their bodies, their dietary is naturally of the lowest value They cultivate a moist, permeable, often unfertile soil, which is in the main unsuitable for the higher cereals (wheat, gram, rice and dal), hence maize (junhri) and sweet potato (ganji) form in the winter and millet in the hot weather the main elements of their diet There is usually only one meal a day Even if higher value foods are cultivated they are exchanged for other life essentials

(8) *Salt*—The salt consumed is that known as Sambhar, obtained by evaporating sea-water and not sendha (rock-salt) which is more expensive After food famines, goitre seems to increase and new foci seem to appear

(9) *Associated diseases*—1 *Malaria*—Owing to high sub-soil water and warm moist climate of the endemic areas malaria is very common, whether in the hill valleys, at the foot-hills or at river junctions

2 *Round-worms*—For the same reasons with the easy pollution of the usual shallow drinking-water wells, round-worm infection is universal, up to some 60 per cent of the entire population being infected Indeed the doctors of the endemic area commonly state that practically every child is infected

3 *Hydrocele and elephantiasis*—Few cases were observed in Gonda and Bahraich, but more were found in the Basti marsh tract associated with endemic goitre Here some 20–25 per cent of the people suffer from hydrocele mostly young adults over 15 years of age Elephantiasis of the scrotum and legs is also found in the Basti marsh tract in about 3–10 per cent of the people, the villages nearer the Gogra being most affected This disease appears

to have been comparatively recently introduced (50 years back) and to be spreading It is a popular saying that men with goitre will not suffer from

CHART 1

Showing monthly goitre and malarial attendance at the Banpur Hospital, 1925-26

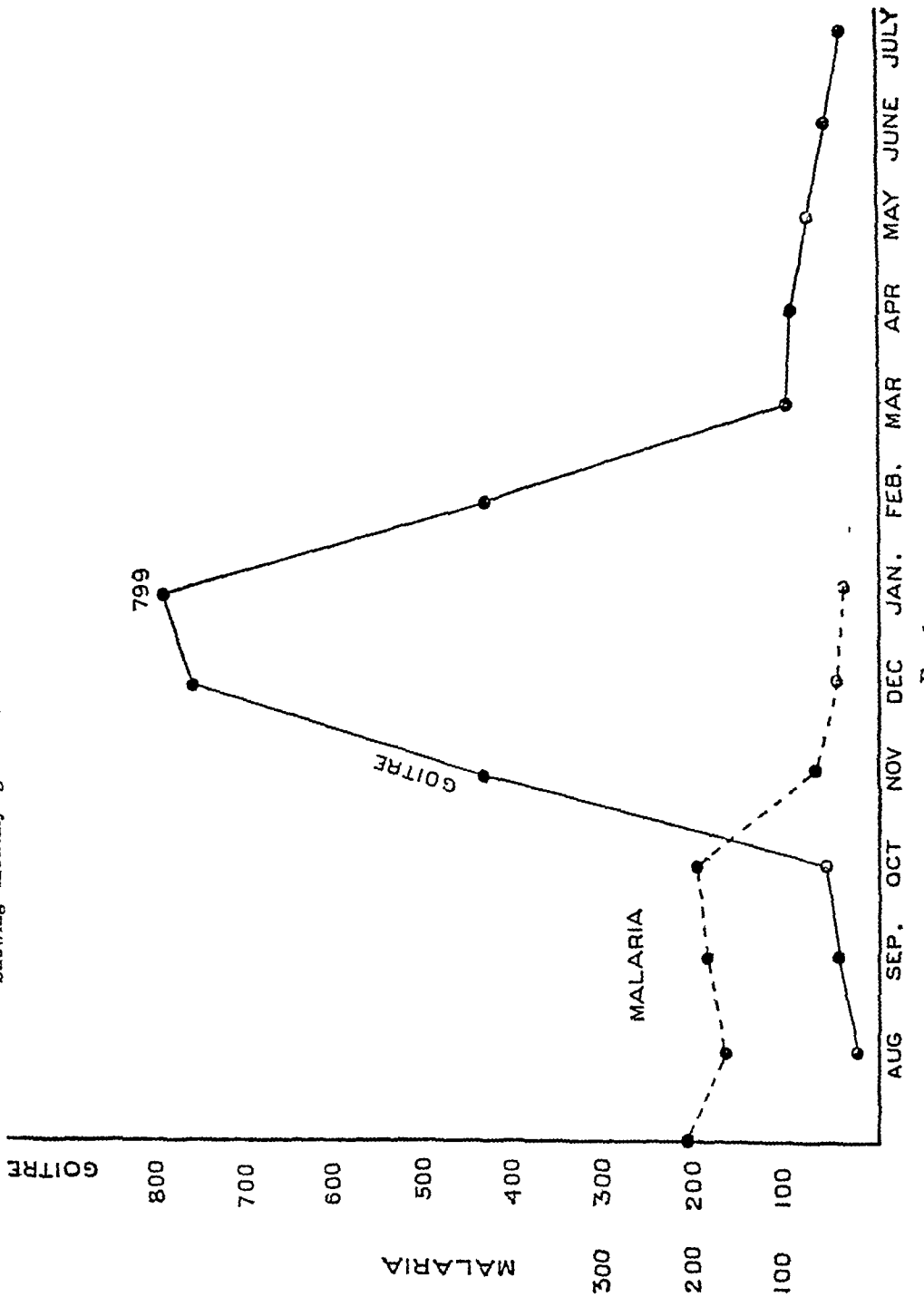


Fig 1

hydrocele, nor will hydrocele appear in goitrous families, villages or areas Certainly this seems to apply to Gonda and Bahraich and also in the dry sandy Fyzabad district south of the Gogra where goitre (except imported cases) is unknown, but where hydrocele is very common

4 *Plague and cholera* are also reported as endemic in the Tarhai districts of Gonda and Basti Such endemic diseases with the extremely poor diet consumed cannot but render the population more susceptible to goitre, as indeed to any other disease

(c) *The endocrine disturbance found in the endemic area*

(a) *Usually nil*—A goitre of itself usually has no effect on the physical and mental characteristics of the individual affected It would seem that sufficient thyroxine is usually produced by the disturbed gland to satisfy the individual's requirements

(b) *Hyperthyroidism*—Exophthalmic goitre, which trained observers are not likely to miss, was not observed in the endemic areas though especial observation and inquiry was made for this disease Exophthalmic goitre is very rare amongst Indians In eight years' experience at King George's Medical College I have only seen two undoubted cases, one male and one female, though I have seen more cases acquired in India amongst Europeans and Anglo-Indians My colleges have a similar experience

(c) *Sub-thyroidism*—In the worst endemic areas, however, there is a very low mental standard amongst the whole population Distinct evidence of adult myxœdema may be found in (1) the atrophied skin covering the thick myxomatous subcutaneous tissues, with supra-clavicular pads, (2) the thick puffy face, lips and tongue, (3) the lowered body metabolism, sluggish movements and slowed circulation, (4) dull mentality with deficiencies of speech and hearing, many of the latter, may be returned as mental defects

(d) *Cretins*—Cretins are born of women with partial thyroid deficiency or with signs of more fully developed myxœdema In the areas of greatest endemicity (e g, in Gorakhpur on the alluvium of the Gandak and in Gonda on the alluvium of the Tarhai) such cretins are naturally mainly congregated Cretins may be grouped into three classes (Sardinian Commission) according to the degree of their mental capacity First class (or true cretins) who are entirely devoid of any intellectual faculty and are without power of speech or reproduction Second class (or semi-cretins), whose intelligence is confined to satisfying their bodily wants, but who can speak in a rudimentary language and can reproduce Third class (or cretinoids) with sufficient intelligence to be trained to some simple work Cretins of all classes are plentiful in all bad goitre areas in the U P All cretins are born of sub-thyroidic parents, and can at once be picked out from groups of villagers by (1) their dwarf size, (2) idiocy, (3) pig-like features, and sometimes with (4) large head, massive trunk, small extremities, small sex organs, (5) coarse swollen thick skin, lips, eyelids and tongue Plate LVII, fig 1 was secured in a Burma endemic centre,

and fig 2 in the Padrauna area These latter cretins were of a thin type I was interested in their lowered carbohydrate tolerance The fasting blood-sugar and blood-sugar curves following 50 grammes of oral glucose of 5 cretins which was kindly investigated by Rai Bahadur Captain J G Mukerji, worked out as under —

TABLE I

Cretin	Fasting	Hours after 50 grammes oral glucose			
		½	1	1½	2
1	0 08	0 11	0·13	0 13	0 10
2	0·10	0 11	0·14	0·18	0·15
3	0 10	0·11	0·13	0·12	0 08
4	0 10	0·11	0 14	0·10	0·10
5	0 10	0 11	0·09	0·09	0·10

Three cretins were subjected to an oral glucose test of 200 gs of glucose but excreted no sugar thereafter

(*e*) *Congenital deaf-mutes* —The close connection between deaf-mutes, cretins and goitre is well known, but since the census records the distribution of deaf-mutes in each province of India, and since these figures are utilized later as an indication of the distribution of goitre in India, it is desirable to produce definite evidence of this close connection In the U P census report for 1901, the following figures are given for the Gonda Tehsils —

TABLE II

Goitre cases attending dispensaries

	Tehsil	Total number	Per 10 000 population	or as	Deaf-mutes per 10,000
1	Gonda	19,385	509	5 6	4 7
2	Tarabganj	29,971	821	9·1	9 3
3	Utraula	5,899	90	1 0	2 7

Allowing for the large percentage of non-goitrous Nepalese who come to the Utraula dispensary with ordinary complaints the tehsil ratio for goitre and for deaf-mutes is practically identical This is interesting evidence But similar figures could be presented for any sufficiently endemic area in the U P, for goitre, cretinism and deaf-mutes everywhere occupy the same areas of the highest endemicity

Again deaf-mutes are frequent in families whose other members are cretins and whose parents have goitre, and these three diseases tend to recur in the affected families Healthy parents coming to an area of high endemicity produce children with goitres and also produce cretins and deaf-mutes, whilst if the parents remove to untainted districts they seldom produce diseased children

In the next two sections the distribution of deaf-mutes according to the census returns can be compared with the distribution of goitre according to our own surveys These distributions will be found to be coterminous In India therefore the distribution of congenital deaf-mutes may be taken as an index of the distribution of severely affected endemic goitre areas

SUMMARY

1 The clinical history of the neck swelling and of its various ætiological factors in the United Provinces are described The common onset of the goitre at puberty amongst ignorant cultivators especially during or after the rains is noted The sex, family and dietetic conditions and the associated endemic diseases are recorded

2 No endocrine disturbance is produced by the usual endemic goitre No case of exophthalmic goitre was observed in the endemic areas and is in any case very rare amongst Indians The disease group of cases of simple goitres, sub-thyroidism, cretins and of congenital deaf-mutes occur together in the same endemic areas

3 The distribution of congenital deaf-mutes may be taken in the worst areas as an index of the distribution of goitre in India

II DISTRIBUTION OF CONGENITAL DEAF-MUTES IN THE UNITED PROVINCES

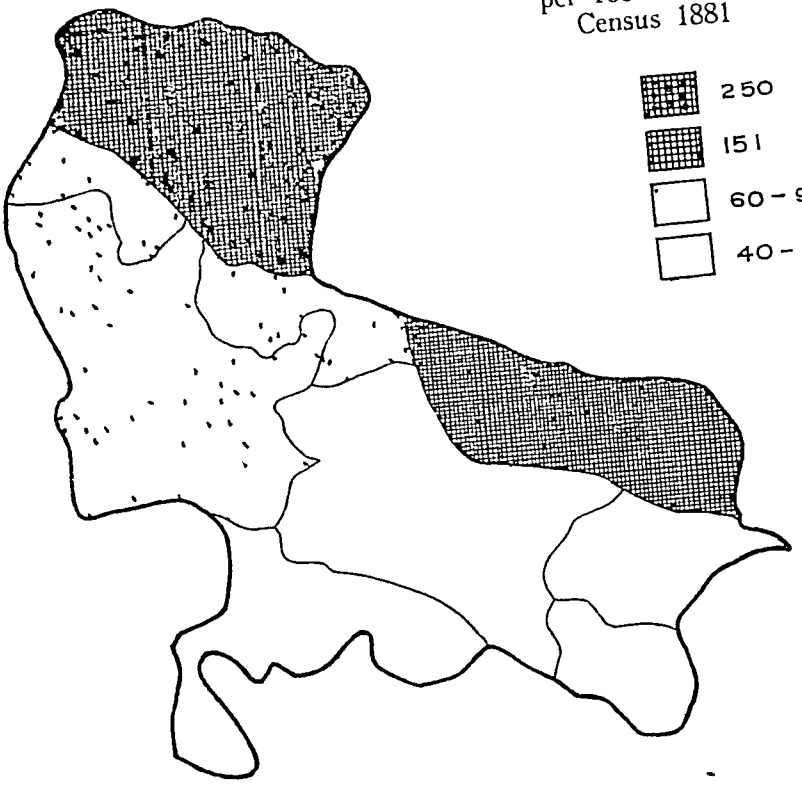
On each of the five attached maps (Maps 1 to 5) I have charted the distribution of congenital deaf-mutes per 100,000 of male population according to districts in the United Provinces for the census years 1881, 1891, 1901, 1911 and 1921, whilst Map 6 provides a named key to the districts and rivers of the preceding five maps The areas most affected at each census are marked in different shadings

Areas affected—At each census there have been two areas mainly affected (1) The Himalayan Tract consisting of the districts of Tehri State, Garhwal, Almorah and Naini, and (2) a sub-Himalayan tract consisting of the districts of Bahraich, Gonda, Basti and Gorakhpur

Decrease in numbers—It will be further noticed that the number of congenital deaf-mutes recorded have diminished remarkably at each census so that there were approximately half the number recorded at the 1921 census as at the 1881 census This decrease is in part apparent and in part real Apparent because in the census of 1881 and 1891 only deaf persons were numbered, whereas subsequently it was laid down that the person must be both deaf and dumb, and in addition only those deaf and dumb *since birth* were to be included The fact that the largest proportionate decrease after 1891

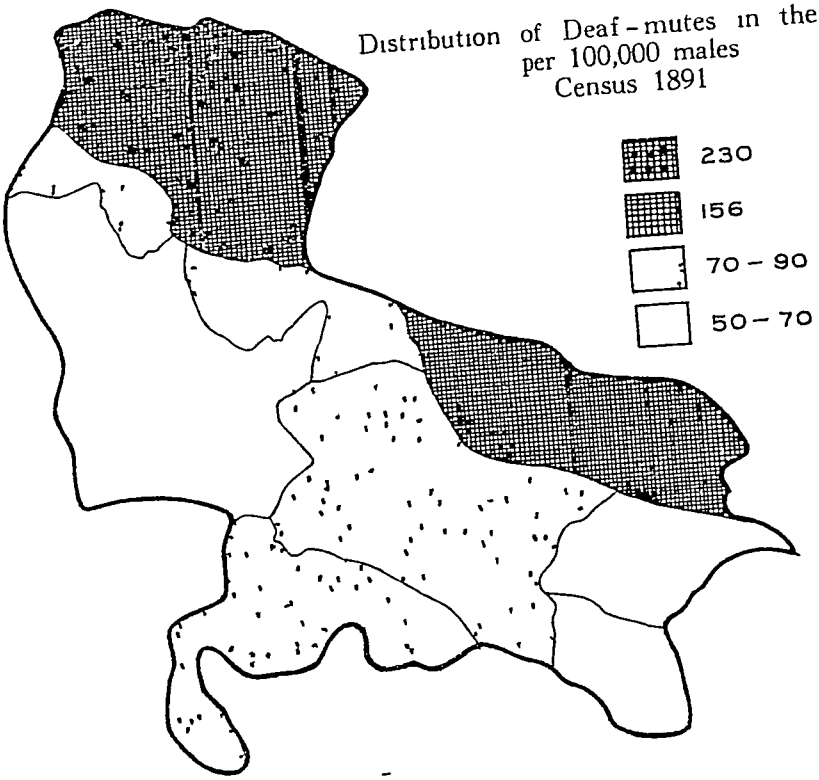
MAP 1

Distribution of Deaf-mutes in the U. P
per 100 000 males
Census 1881



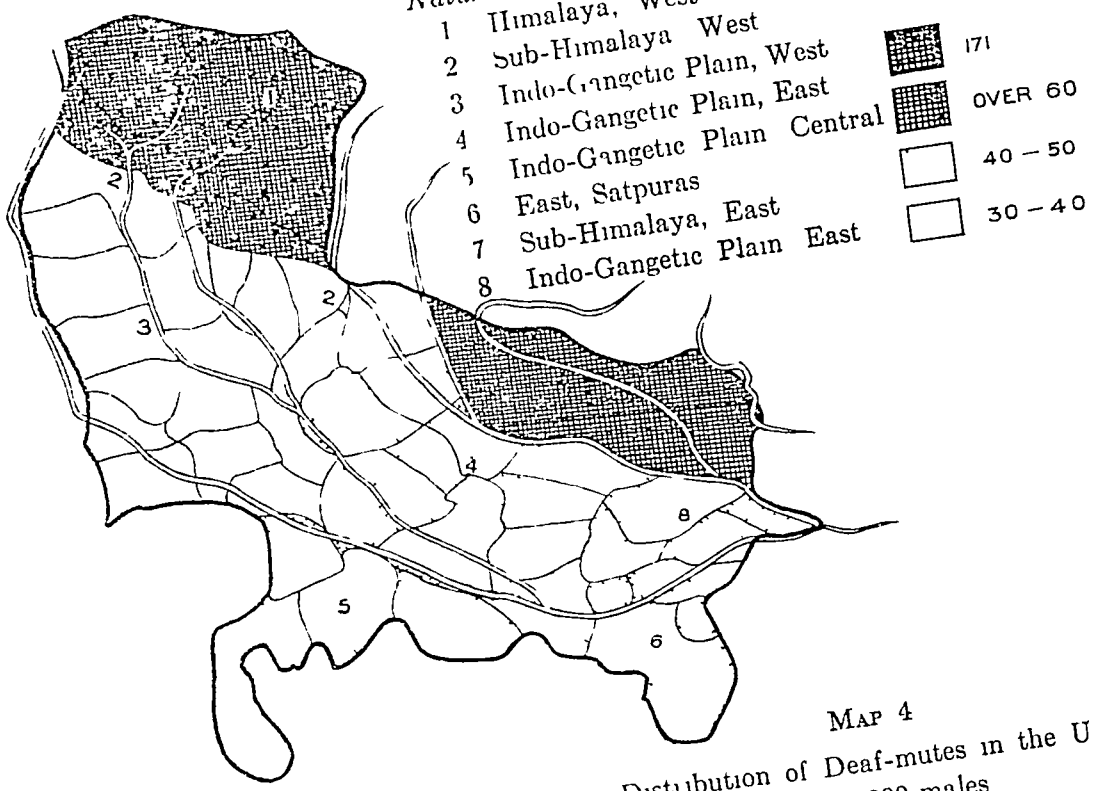
MAP 2

Distribution of Deaf-mutes in the U P
per 100,000 males
Census 1891



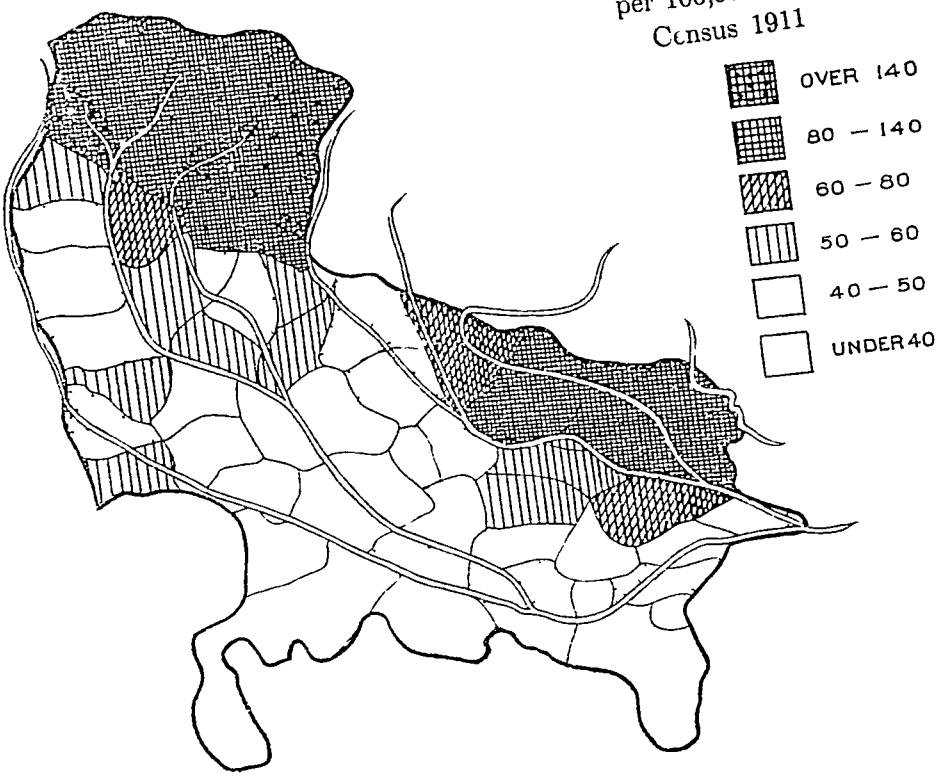
MAP 3

Distribution of Deaf-mutes in the U P
per 100,000
Census 1901



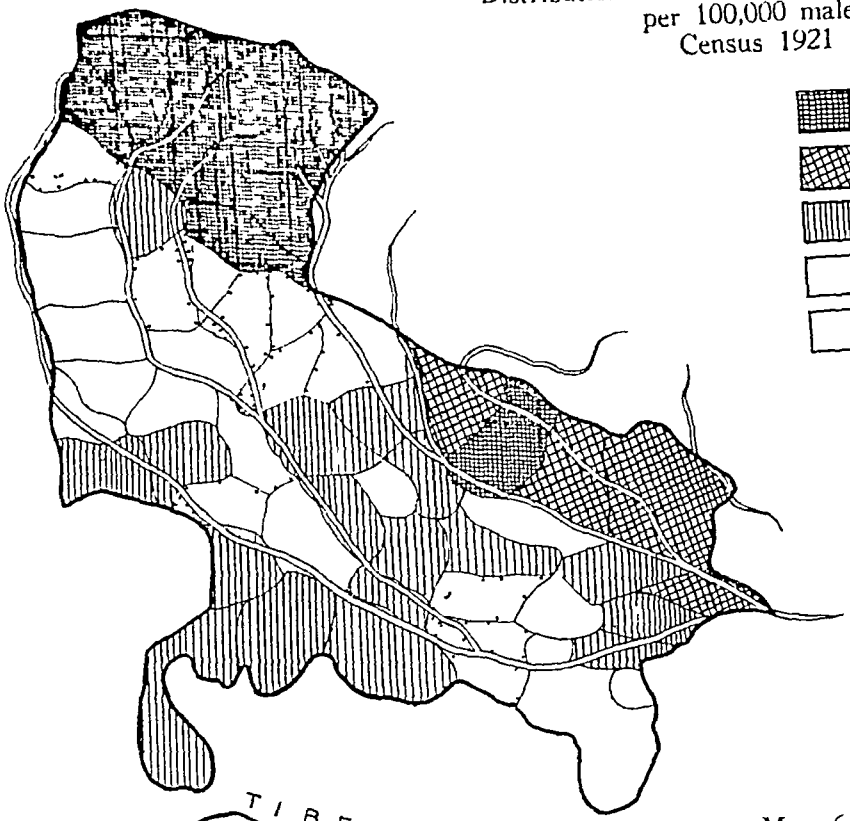
MAP 4

Distribution of Deaf-mutes in the U P
per 100,000 males
Census 1911



MAP 5

Distribution of Deaf-mutes in the U P
per 100,000 males
Census 1921



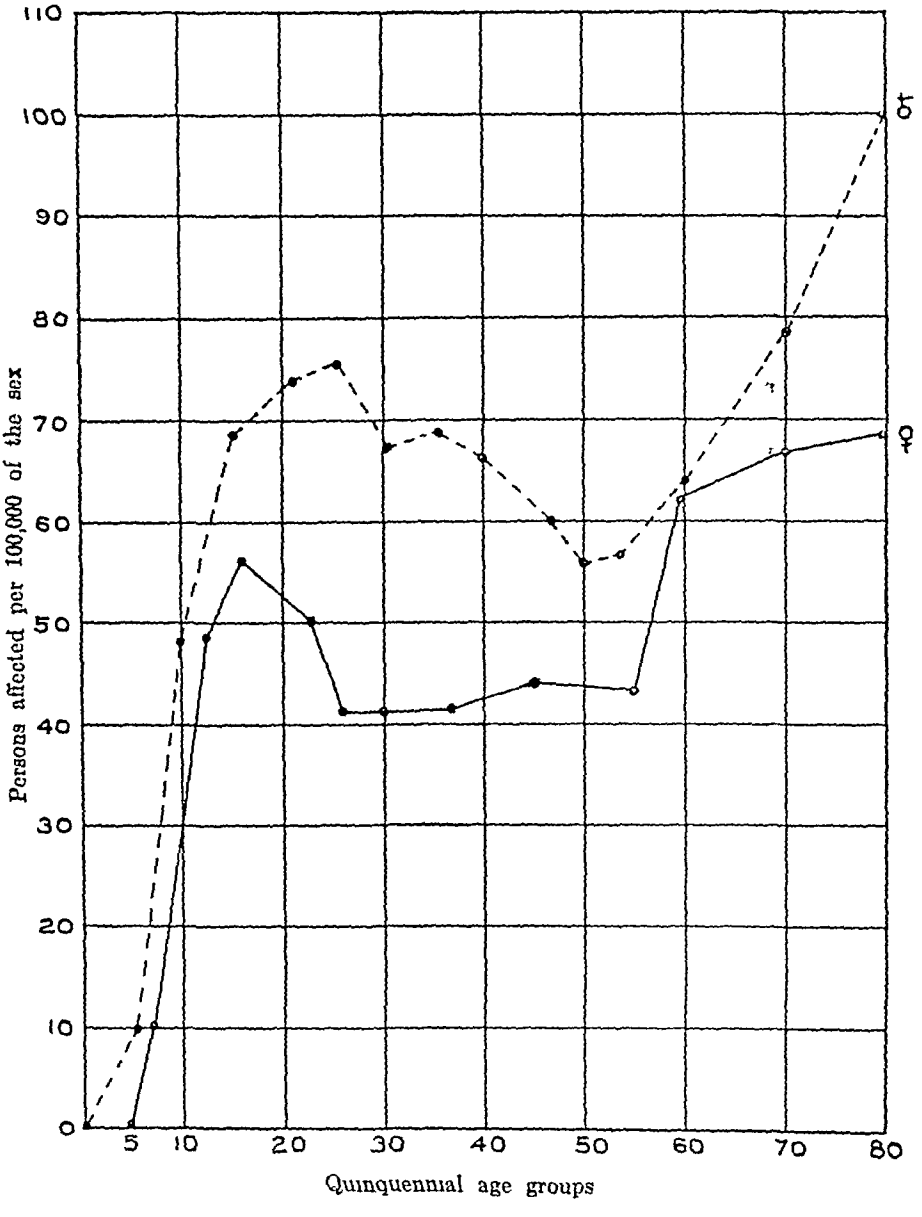
MAP 6

Key Map to district and rivers of the United Provinces

was in the age period over 60 substantiates this point In 1911, there were about one quarter lakh of congenital deaf-mutes in the province

CHART 2

Distribution of congenital deaf-mutes by age
Census 1921
(Census of 1911 is very similar)



Age distribution—The distribution of deaf-mutes by age in the United Provinces according to the 1921 census is shown in Chart 2 and is very similar

to that of 1911 There is a vast underestimate of congenital deaf-mutes in the 0–5 years group For parents hesitate to report their child as a deaf-mute until all hope is lost, preferring to believe as long as possible that speech and hearing is merely delayed Deaf-mutes are not long lived so that the numbers charted at the higher age periods should be increasingly small, but the increases shown in the chart over 50 years of age obviously include many cases of deafness arising in old age

III Distribution of endemic goitre in the United Provinces, and its relation to special drinking waters and to special soils of high calcium-content

Introduction

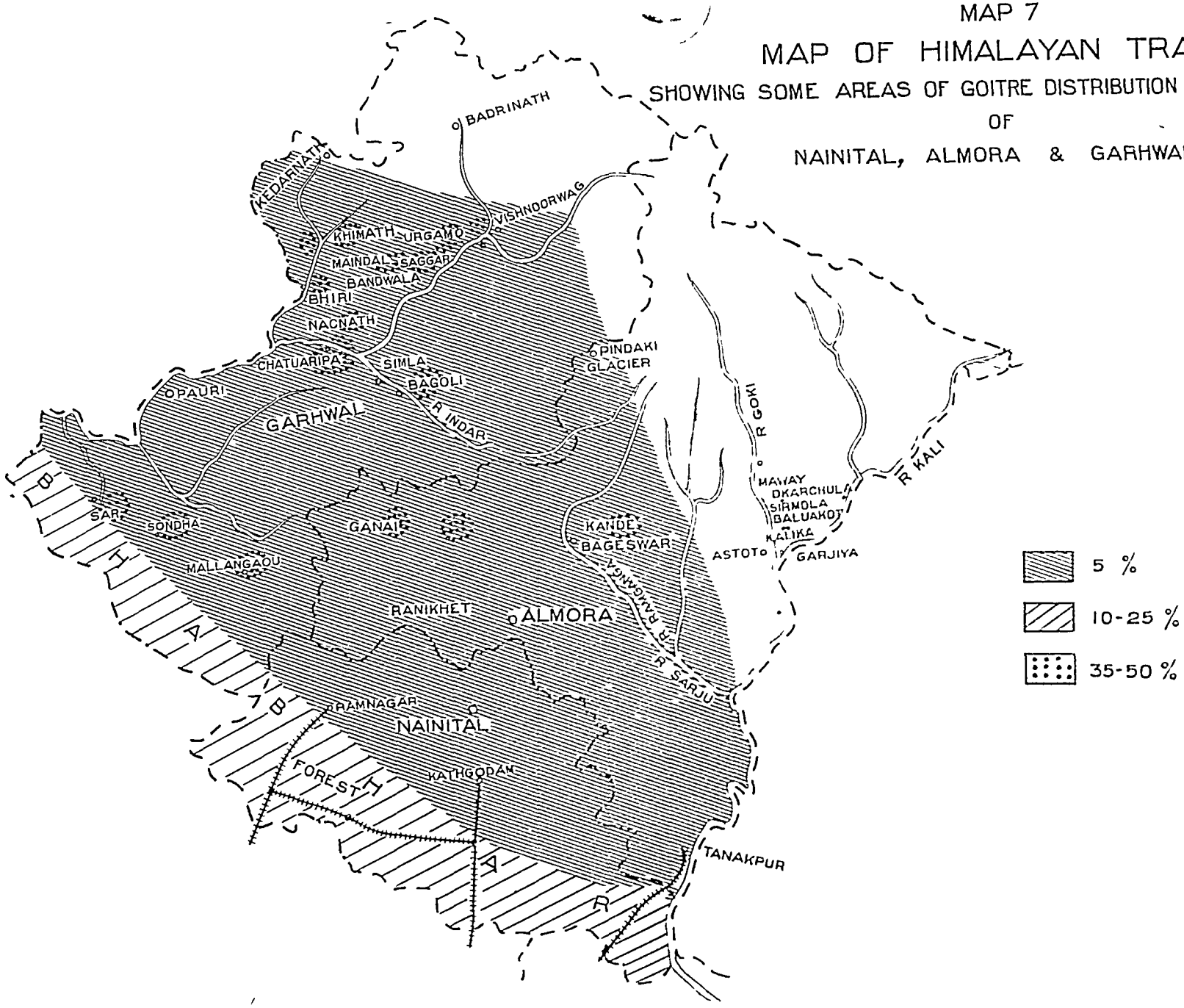
1 *The four endemic areas*—The distribution of congenital deaf-mutism in the United Provinces as set out in the preceding section into (1) the Himalayan Tract of Tehri, Garhwal, Almora and Naini and (2) the sub-Himalayan tract of Bahraich, Gonda, Basti and Gorakhpur indicated that the endemic areas of goitre will also be found in those tracts Information generally available in the United Provinces supported this view and additional evidence was afforded by Civil Surgeons in reply to a circular letter as to the frequency of goitre in the respective districts of the province Investigations were therefore concentrated in these tracts In the sub-Himalayan area two tracts, which merit separate consideration, were noted, viz, the marshy Tarhai tract of Bahraich, Gonda, Basti and the damp Padrauna tract of Gorakhpur To these two tracts may be added a third tract along the Terai foot-hills, which is an area of minor endemicity only, with which may be considered the upland (Upatharai) tract

2 *Goitre and non-goitrous waters*—The common belief amongst villagers is that the cause of goitre lies in the water, and this is supported by a large mass of sound evidence collected by many observers, medical and others, investigating goitre in its endemic areas Wherever for instance, one portion of a village was more markedly goitrous than another portion, it was found that villagers took their water from different water-supplies, one of which was recognized as being more goitrous-producing than the other Some water-supplies indeed are known as 'goitre' wells or 'goitre springs' by villagers in that the drinking of such water causes goitre to develop whilst other rivers, wells, or springs are known not to cause goitre Some of the latter waters indeed may establish a reputation for curing goitre, for if persons with early soft goitre drink only from such supplies the goitre will disappear In such a case the pure water operates in a similar manner to that of a good water-supply drunk by a person who leaves an endemic area and whose goitre disappears on this account Such 'goitre wells' and 'goitre-curing wells' have had their peculiar properties tested by generations of villagers

MAP 7

MAP OF HIMALAYAN TRACT

SHOWING SOME AREAS OF GOITRE DISTRIBUTION IN THE DISTRICTS OF NAINITAL, ALMORA & GARHWAL



3 *Calcium-waters*—Moreover many villagers recognize that it is 'chuna' water containing a large excess of lime and coming from lime rocks that is the peculiar cause of goitre Such goitre-producing water is recognized by villagers as having lime-containing properties by the following characters (1) hardness, (2) 'heavy' consistency, (3) 'pricking' (astringent) taste, (4) lime 'smell,' (5) remaining warm in all seasons whilst good (goitre-free) water keeps cool (this is an outstanding comment of the people), (6) high lime content, so that (*a*) when boiled a fine chalky deposit is left on the vessel, (*b*) when concentrated in the hot weather, the water becomes milky and deposits white on a brass lota turning the latter reddish, (7) persons drinking such water develop goitres, (8) if such drinking water be changed for good (non-goitrous) water, small soft goitres will disappear Hence drinking water containing a large amount of lime is regarded as the cause of goitre by many villagers in these endemic areas

4 *Differing factors in the four endemic areas of the U P*—Each of the big endemic areas of the United Provinces requires separate consideration for the main factors producing an excess of calcium in the water-supply of each varies The chief source of goitrous water in —

(*a*) *The Himalayan Tract,* are the hard (calcium) springs from soft lime rocks

(*b*) *The Padrauna Tract,* are shallow well-waters supplied from a damp alluvial soil of extremely high calcium-content

(*c*) *The Foot-hill Tracts,* where there are many small mountain streams running over water-worn limestone boulders and mountain debris

(*d*) *The Marshy (Tarhai) Tract,* where somewhat similar conditions to the Padrauna area exist but where the many mountainous tributaries flowing into the north bank of Gogra river especially affect the sub-soil water level and local conditions

Each of these four areas may now be considered in greater detail

(*a*) *In the Himalayan tract*

5 *Goitre prevalence*—Map 7 sets out the goitre distribution as observed in the Himalayan tracts of Tehri, Garhwal, Almorah and Naini The map is far from complete Owing to several circumstances this area could be surveyed only in part Unlike the plains where endemic goitre is found in large areas, in the hills goitre is scattered in different and often not adjacent villages These endemic villages are situated in the hot moist river valleys where maize is often freely cultivated Villages over 5,000 feet in cool climates appear free Some 35–50 per cent of the population of the endemic villages are affected Some 3–5 per cent of the population in endemic villages are deaf-mutes Cretins are also found One small hill area was observed in which goitre is especially endemic, viz, that area between the two rivers Kali and Gori, which later become the river Gogra running south of Bahraich For the

past 20 years there has been a general decline in the goitre rate and new cases of under 15 years are now not commonly met with, far less frequently than in the endemic plain areas Of the cases seen 60 per cent were between 15 and 45 years and only 30 per cent between 6 and 14 years After famines which are not infrequent in the hills, inhabitants of new villages are apt to show thyroid enlargement Goitre is also prevalent in the foot-hills (Bhabhar) of the Naini and Garhwal districts to a rate of some 10–25 per cent The Bhabhar soil is friable and porous, the upper layer being washings from the hill-sides and consists of a thin covering of alluvium over boulders and shingle The sub-soil water is at 8–10 feet The soil is moist and numerous nullahs and streams drain off the hill-water

6 *Water-supplies*—The water-supply of these hill villages is by mountain springs direct, or by a small tank into which such springs trickle Where there are no springs, open artificial channels are used to bring water from a high level supply over some distance In the Bhabhar which is very dry, water flowing down the mountain slopes (always small in quantity) is stocked in shallow wells for drinking

7 *Calcium-content*—Hard (lime) water springs are believed by the hill villagers to be the cause of their goitres In every village where goitre was endemic Dr Lal traced the water-supply during this investigation to calcium rocks There are two varieties of limestone rocks commonly found, (1) a dark hard variety and (2) a soft white variety Both varieties are burnt in kilns to prepare lime for building The soft white variety is the one found associated with calcium springs A little earth is deposited on the stones at the mouth of such springs in which weeds grow Later some calcium is deposited in the meshes of these weeds, gradually more and more calcium is deposited until large blocks, some in most artistic designs, are formed Such rocks are known by villagers as 'growing rocks' as the new lime formation with each annual deposit makes the rocks bigger and bigger Such 'growing' rocks are sometimes dug out by contractors for building lime These rocks are very porous and water highly charged with calcium in solution trickling through them freely evaporates during the hot weather in the hot valleys thus adding to their rapid growth Such limestone rocks are closely associated with water highly charged with calcium (hard water)

8 *Examples*—Examples of the association of calcium-water producing goitre in the Himalayan tract may now be quoted —

(1) In Gorjia in Almorah the water-supply is so heavily charged with calcium that it becomes milky during the hot months Sixty per cent of this village are goitrous

(2) Baluakot and Nangtai are adjoining villages in Almorah with all conditions of life the same except that the former has a hard water from calcium rocks as a water-supply with 45 per cent of goitres, whilst the latter has a soft water-supply and is goitre-free Such a fact explains the apparent haphazard distribution of goitre from one village to another There is no large area where

all drinking water comes from calcium rocks and hence no large localized distribution of goitre, as in the plain areas

(3) Many villagers can tell by the characters already mentioned whether any given water contains lime in excess or not and have come to associate the former with the cause of goitre Hence when they wish to be goitre-free they aim at changing to a non-goitrous water Village Neoli in Almorah used to be famed for its goitres, but now the goitre rate is only 25 per cent, and no adolescents have acquired goitre since the water-supply has been changed to a goitre-free source

(4) Village Urgam in Garhwal and Dharchula in Almorah are examples of one section of a village taking its drinking water from a calcium rock source and suffering heavily with goitre whilst another section drinks from a pure spring and remains goitre-free

(*b*) *In the Padrauna Tehsil of Gorakhpur*

9 *Goitre distribution of sub-Himalayan area*—Map 8 charts the approximate goitre distribution in the sub-Himalayan tract which lies between the river Gogra to the south and the Himalayas to the north and consists of the Bahraich, Gonda, Basti and Gorakhpur districts The areas of high goitre endemicity are not equally distributed over all four districts but are concentrated in two main areas The first of these is the Padrauna Tehsil immediately west of the great Gandak and the second being the low-lying moist (Tarhar) tract of Bahraich, Basti and Gonda A minor area is that north of the Terai (foot-hill) tract

10 *Bhat soil*—It is the universal belief amongst villagers founded on generations of experience that goitre is due to drinking water from wells sunk in special soil known as Bhat soil which is spread over 75 per cent of the Padrauna Tehsil For all practical purposes the distribution of goitre in the Gorakhpur district follows almost exactly the distribution of this Bhat soil, whereas the soil of the adjoining Mahrajganj Tehsil is known as Bangar soil and is practically goitre-free (5 per cent) A strip of Bangar soil runs into the Padrauna Tehsil and the goitre percentage on this is also 5 per cent only Hence the characteristics of this Bhat soil and its points of contrast with Bangar soil demand the closest scrutiny

Bhat soil consists of alluvium brought down from the Himalayas by the great Gandak river and spread over the Tehsil during the flood times when the numerous small affluents of the Gandak become distended and overflow their banks The water penetrates the very permeable Bhat soil, leaving at each flooding a fresh deposit of the alluvium and salts brought down from the hills and added to the former alluvium Bhat soil is about 3 feet deep deposited on layers of sand It contains small limestone rocks It is mainly a limestone soil, being therefore very rich in calcium (32 per cent) which may be compared with the calcium-content of Bangar soil (2 per cent) which is stiffer with more

clay and less sand in its composition Bhat is remarkably white, the whiteness being non-crystalline but of a powdery white consistence, both of which features are apparently due to its high calcium-content The soil is so friable that oxen are not required for ploughing, the light porous friable soil being readily turned with a hand bamboo shear It is so friable that the making of unbricked wells is most difficult The water from the wells in this Bhat soil is very hard (far harder in Padrauna than in Gorakhpur) from a high calcium percentage rate For crops Bhat soil is only a medium soil, for though all crops can grow on it they can only produce a poor supply but no expense is incurred for cattle for ploughing Bhat soil is very permeable and extremely retentive of moisture with a high sub-soil water-level at 10–12 feet all the year round It is an aphorism in the area not to sleep on this damp Bhat soil The marshy nature and presence of a large number of small streams and nullahs is another feature of this goitrous soil In the Padrauna Tehsil then we have a remarkable example of the endemic goitre being practically limited to the distribution of a special alluvial soil whose striking characteristic lies in its high calcium-content of 32 per cent, some hundredfold the normal percentage of calcium in soil This calcium is brought down in the hill streams from the limestone mountain rocks, and is deposited with the alluvium in the marshy soil, which possesses a very high sub-soil water-level The shallow wells in this soil thus become readily filled with a hard water of high calcium-content

11 *Goitre rates*—The Padrauna Tehsil lies some 400 feet above sea-level Rural areas are far more affected than the only town (Padrauna) where the water-supply has been secured good A village goitre rate may fluctuate over a series of years, an improvement being attributable to an improved well supply The disease appears to be on the decrease Deaf-mutes are common Amongst 600 persons with goitre six cretins (1 per cent) were counted The great Gandak forms the eastern boundary of the endemic area, and the little Gandak the western boundary The latter is usually dry except during the rains when also many small shallow lakes are formed between the two

12 *Water-supply*—Well-water is used for drinking, and the wells are usually very shallow as the underground water-level is very high At times during the rains the sub-soil water-level and the surface water become indistinguishable and the saying goes that 'cattle drink water from the wells' Although the great Gandak is responsible for the Bhat soil and hence indirectly for the endemic goitre of this area, yet those living on either of its banks and who drink its water escape goitre, whereas the incidence of the disease is high near its affluents, e g, the Bansi running through the areas of the Bishanpore and Tarsi Sujan police stations The reason lies in the great strength of the Gandak stream from which suspended matter quickly sediments Well-water of many wells were kindly analysed by the Public Health Department The following goitrous water from village Khada in Gorakhpur is an example Solids, total 90, fixed 46, volatile 44 Total hardness 32, calcium 24, both in

parts per 100,000 Iodine nil (In no waters goitrous or otherwise in the U P was iodine noted) Nitrites and nitrates present

13 *Climate and inhabitants*—The climate is very moist, the rainfall (50–70 inches) being higher than in any other plain district of the province The Tehsil is densely populated (700 per square mile) practically all being engaged in agriculture Very backward, poor and under-fed, the villager of the endemic area is appreciably inferior in capacity and intelligence to those elsewhere in the district He is indeed so poor as to be unable to afford sufficient clothes to cover his body His diet is mainly rice and maize The salt is of sombre quality (evaporated sea-water), imported from outside No difference could be detected in the dietaries of those living in the affected and non-affected villages

(c) *In the foot-hill (Terai) and plateau (Upaihar) tracts of Bahraich, Gonda and Gorakhpur*

14 Three of the sub-Himalayan districts of Bahraich, Gonda and Basti fall naturally into three transversely running tracts known as —

(1) The foot-hill (Terai) tract
(2) The plateau (Upaihar) tract
(3) The low-lying moist (Taihar) tract

The latter is an endemic area of high degree, the former two areas will also be considered in this section

15 *The sub-mountainous foot-hill (Terai) tract*—This tract lies between the Himalayas to the north and the river Rapti to the south The area is full of small nullahs, dry in the hot weather but which serve to draw off mountain water in the rains and which then form many swamps To the extreme north there is a line of forest land, where the heavy clay forming the soil of this area gives ways to sandy clay which is interspersed with water-worn limestone boulders and lime kanker from the mountains Water in the Terai is drawn from the shallow wells which are, however, very few in number especially in the forest area When these run dry, as they often do, the people use the small hill rivulets for drinking Along the Rapti river the villages are goitre-free, nor does the disease become intense until about one mile from the forest border is reached, where the villages commence to show an increasing prevalence from 3–5 per cent The worst villages, some of which show a rate of 30 per cent, are situated on the edge of the hill-rivulets The disease in general is not severe here It is said to have only been first observed some thirty years ago The size reached is seldom bigger than that of a tennis ball Big goitres are usually imported cases No deaf-mutes or cretins were observed The common food is maize (junhri)

16 *The plateau (Upaihar) tract, 390 feet*—This tract is separated from the foot-hill tract on the north by the river Rapti, and from the low-lying moist Taihar tract to the south by the rivers Sarju, Tehri and Kuwano The tract is a slightly raised plateau The soil is a stiff clay, unsuited to maize

but the grain harvests are rich, and the Uparhar is the most flourishing part in each district Wells are plentiful The tract is almost free from endemic goitre, such cases being usually imported from the south Such old-standing goitre were hard and larger than a tennis ball Only two of some two hundred villages examined showed a general prevalence, one (Pandit Purva) only a few miles from Gonda city on the Tarhar boundary possessed only seven houses, one well, 25 persons with 50 per cent goitres Several of this village were partially deaf Two deaf-mute brothers had a goitrous mother

(*d*) *In the low marshy (Tarhar) tract of Bahraich, Gonda and Basti*

17 *Goitre rates*—Endemic goitre is not spread evenly over this marsh tract, which lies some 310 feet above sea-level south of the Uparhar edge and north of the Gogra river The goitre rates vary under three separate conditions Firstly, the incidence increases rapidly southwards from the district towns of Bahraich, Gonda and Basti, the percentage rising in different areas from 10–80 per cent Secondly, the goitre rate again decreases along a narrow strip of land bordering the north bank of the Gogra, where there is little goitre Thirdly and by far the most markedly, the goitre rate north of this narrow strip along the northern bank of the Gogra is greatly influenced by an area of land being enclosed between two rivers, e g, (1) in Bahraich, at the junction of the Chandra and Gogra river (50 per cent), (2) in Gonda, between the Tehri, Sarju and Gogra (70 per cent), and (3) in Basti, between the Manwar, Kuwano and Gogra (50–70 per cent) Such areas are comparable to other endemic areas between rivers as (4) that between the Kali and Gori rivers in Almorah (40 per cent) and (5) between the two Gandaks in Gorakhpur (50 per cent) Of all endemic areas that lying between the Tehri, Sarju and Gogra of the Gonda district and known as the 'Tirvaha' or land between three rivers is the most severely affected having a goitre rate of some 70 per cent whilst numerous idiots and deaf-mutes are met here Amongst some 5,425 persons in 13 hamlets there were 24 deaf-mutes and 2 cretins, i e, roughly 1 in 250 was a deaf-mute Children with defective speech, deficient hearing and feeble mentality were very common The inhabitants themselves designate the worst villages in this area as 'the abode of fools'—and it is a popular axiom that human intelligence diminishes with length of residence in this area The oldest inhabitants state that goitre has been prevalent in this area for generations, long beyond human recollection

18 *Marshy soil with high sub-soil water*—The soil of these areas, like the Bhat soil of the Padrauna Tehsil, is alluvial and is kept in a high state of saturation with a high sub-soil water (about 10 feet in the dry weather and 4 feet or less in the rains) by the confluence of a large tributary with the Gogra The area is beset with numerous small streams and standing water expanses which vary in extent with the season During the rains the water of the tributaries of the Gogra with their limestone washing from the hills are forced backwards into the streamlets From these the tract is very liable to

inundation and remains waterlogged, the soil receiving fresh deposits of alluvium and of calcium The flow of the sub-soil water is naturally greater in the upland than in the marsh tract The climate is naturally humid The drinking water is mainly from shallow wells

The soil is sandy or sometimes a porous loam, but again like that of the Padrauna Tehsil, it is very friable and permeable so that ploughing with oxen is unnecessary, a small hand-spade only being used The chief crop is maize and sweet potato but large unfertile stretches remain uncultivated The soil, like that of the Padrauna Tehsil, has a very high calcium rate (9 per cent) being some thirtyfold above that commonly found (0 3 per cent) The following is the table showing the calcium-carbonate in various samples of soils of all tehsils of six districts kindly analysed for me by the Director of the Agriculture Department, U P —

TABLE III

Showing the amount of calcium-carbonate in soils of all tehsils of six districts

Name of district	Tehsils	Calcium-carbonate
Gorakhpur	Gorakhpur	0·25
Do	Padrauna	39·36
Do	Deoria	0·27
Do	Hatha	0·23
Do	Mahrajganj	2 70
Do	Bansgaon	1 39
Basti	Basti	0·27
Do	Khalilabad	0·23
Do	Bansi	0·73
Do	Domariaganj	0·25
Do	Harraiya	0·29
Azamgarh	Azamgarh	0 38
Do	Nizamabad	0·32
Do	Ghosi	0·65
Do	Sagri	0·34
Do	Muhammadabad	0·32
Do	Ahraula	1 98
Gonda	Gonda (Tarhar)	9·13

TABLE III—*concld*

Name of district	Tehsils	Calcium-carbonate
Gonda	Tulsipur (Upazila)	0·29
Do	Tulsipur (Tarbar)	5·83
Do	Gonda (Upazila)	0·29
Do	Utraula	0·20
Bara Banki	Bara Banki	0·18
Do	Ramsanehi Ghat	0·27
Do	Haidar Garh	0·70
Do	Fatehpur	0·18
Sitapur	Sitapur	0·29
Do	Biswan	0·31
Do	Sidhauli	0·32
Do	Misrikh	0·32

19 *Goitre-producing properties of water-supplies*—There are certain observations of considerable interest concerning the goitre-producing properties of rivers and wells in this sub-Himalayan area which may be enumerated here

(1) Himalayan rivers and streams bring down calcium dissolved and suspended in their waters from the mountainous limestone This is spread over the endemic areas with the alluvium

(2) As soon as the river is well clear of the hills, at a certain point, the goitre-producing property disappears, apparently because the calcium has sedimented

(3) Rivers with strong currents do not give rise to goitre, e g, the Big Gandak has a strong current and hence does not cause goitre, but wells on the bank of the river fed with sub-soil water containing much calcium from Bhat or similar soil, cause goitre So also with the Gogra, Tehri, Sarju and Rapti It is a general rule that villages on the banks of rivers in an endemic area remain goitre-free since the villagers drink the stream-water and escape goitre This explains the strip of comparatively goitre-free areas along the north bank of the Gogra An interesting example was a certain village where a small colony of Christians on the river bank drank river-water and escaped goitre whilst the remaining village population of Hindus and Mohammedans drank from a goitrous well-water and showed a 40 per cent goitre rate

(4) South of the Gogra, in the dry sandy areas of Fyzabad–there is no goitre, because the Gogra tributaries which keep the soil moist and calcium-saturated all flow from the north

(5) At the junction of the Gogra with the Ganges, there are areas in Azamgarh and Balli which have a definite proportion of new goitres though not reaching the high rates of areas north of this river

(6) Periods of water scarcity drive people to goitre-water which itself may have become concentrated from excessive heat and hence is apt to increase the new goitre rate

(7) Pucca deep wells are less goitrous-producing than shallow wells, since a lower water-supply is tapped and the calcium-containing sub-soil water is kept away

(8) New officials and educated persons in endemic areas who are unable to obtain good supplies, boil the water and thus drive off the carbon-dioxide which holds the lime in solution as calcium-carbonate, causing it to precipitate as the well-recognized 'white powder' Hence they escape goitre

(9) As jungle is cleared and water-supplies are improved, the disease decreases

(10) Near Khalilabad in Basti is a village which used to obtain its water from a non-goitrous well in a neighbouring village and had no goitre For convenience a well was dug For some time people who drank this water developed goitre So the well was filled in and water brought from the previous village as before Goitre disappeared

SUMMARY

1 The four endemic areas of the United Provinces are first described The village belief that the cause of goitre lies in the water and especially in calcium-water is emphasized The features by which villagers recognize a goitre-producing water are recorded

2 In the Himalayan tract the goitre distribution is mainly in scattered villages in the hot damp valleys and also in the foot-hills (Bhabhar) The water-supply in the former is mainly by mountain springs and in the latter by mountain streams In every goitre village examined the water-supply was traced to limestone rocks, often the so-called 'growing' rocks Weeds rooted on soft limestone break up the stone and by the carbon-dioxide they produce transform insoluble lime into soluble calcium-carbonate Examples of calcium-waters causing goitre are quoted

3 In the Padrauna Tehsil the goitre distribution follows almost exactly that of 'Bhat' soil which is a powdery friable whitish alluvium about three feet deep brought down from the Himalayas by the Gandak system and containing the enormous proportion of 32 per cent of calcium The drinking water from the shallow wells of this soil naturally contain a high calcium-content (24 parts per 100,000) This Bhat soil is damp with a high sub-soil level Into this 'Bhat' soil of the Padrauna Tehsil a tongue of 'Bangar' soil cuts This soil contains only 2 per cent of calcium, and the goitre rate on it is approximately 5 as compared with 50 rate on Bhat soil

4 The sub-Himalayan area of the United Provinces falls into three natural transversely running tracts (1) The foot-hill (Terai) tract, (2) the plateau (Upaihai) tract, and (3) the low-lying moist (Tarhai) tract, the latter of which constitutes an area of high endemicity

5 The foot-hill (Terai) tract to the north develops into a strip of forest land at the mountain edge with a goitre rate of 10–25 in certain villages drawing their drinking water from hill streams which course over water-worn soft limestone boulders and rocks, and soak through the hill alluvium and debris

6 The plateau (Upaihai) tract is mainly free from the development of fresh goitre cases

7 The marshy (Taihai) tract shows intensely endemic areas where the tributaries from the Himalayas join the Gogra The most endemic of all areas is that lying between the three rivers Tehri, Sarju and Gogra where the goitre rate is 70 per cent and which is known as the 'home of fools' The soil of these areas is intensely damp and the sub-soil water is at a high level especially during the rains Like that of the Padrauna Tehsil the soil is alluvial, friable and porous with 9 per cent of calcium A brief review of the goitre-producing properties of certain water-supplies is given

IV The distribution of congenital deaf-mutism in India

The following information has been collected, in the main, from the *Census Reports for India*–and from each of the eleven Provincial Reports from 1881 to 1921 In all endemic areas, as in Europe and America, the three infirmities of deaf-mutes, cretins and goitre exist side by side in the same areas, and these areas of maximum intensity are ordinarily 'in relation with certain rivers and hills' In 1901, in Champaran of 178 genuine cases of congenital deaf-mutes, 22 were insane, 43 weak-minded (i e, were cretins or cretinoids) and 51 had goitres

2 The rates for deaf-mutes per 100,000 of population are set out with the province, state or agency as a unit in the following table —

Table IV

Proportions in individual Provinces and States

Province, State, Agency	Proportion of deaf-mutes per lakh of population		Numerical order in	
	1921	1911	1921	1911
India	60	64		
Sikkim	176	266	1	1
Kashmir	138	98	2	2

TABLE IV—*concld*

Province, State, Agency	Proportion of deaf-mutes per lakh of population		Numerical order in	
	1921	1911	1921	1911
Burma	90	71	3	10
Punjab and Delhi	89	84	4	4
Central Provinces and Berar	88	47	5	15
Baluchistan	85	80	6	5
North-West Frontier Province	84	96	7	3
Assam	70	76	8	8
Bengal	67	69	9	11
Mysore	60	77	10	7
Bombay	55	61	11	12
Travancore	54	29	12	18
Bihar and Orissa	53	72	13	9
Madras	51	78	14	6
Cochin	51	36	15	16
United Provinces	50	56	16	13
Central India and Gwalior	34	23	17	20
Baroda	28	21	18	21
Hyderabad	27	33	19	17
Rajputana and Ajmer	26	29	20	18
Coorg	12	50	21	14

Thus the provinces at the foot of the Himalayas (excluding the Central Provinces where there appears to be some error in 1921) are shown to be the most affected, except the United Provinces which, on the provincial basis ratio, stands only sixteenth out of twenty-one units But with each province the average of deaf-mutes show extraordinary local variations–and the general low average in the United Provinces is due to freedom from the disease in the densely populated tracts remote from the Himalayas whereas in the Himalayan tract deaf-mutes are as common as in Kashmir, in Sikkim and in Burma which usually show the highest rates

3 The following examples will indicate how greatly the deaf-mute rate varies with local conditions within the province In the Punjab, United Provinces, Bihar and Bengal, deaf-mutism invariably exists in highest degree in and along the foot-hills of the Himalayas

Province	Local area	Deaf-mute rate per 100,000
Punjab	Himalayan Natural Division	257
	Rest of Province	70
Bihar	Champaran District	169
	Provincial Average	72
Assam	Naga Hills	190
	Provincial Average	70
Burma	Shan States	216
	Northern Hill Districts	231
	Open Plains	10

The examples of Assam and Burma indicate that the condition of other hills (apart from the Himalayas) are also productive of deaf-mutism This local variation which is exemplified in the above examples can be identified in every province In *Bengal*, the disease is far more common in Darjeeling and at the Himalayan foot-hills than the rest of the province In *Bombay*, the Sind division, in the *Central Provinces and Bihar*, the Mahratta plain division and in *Madras* the North Arcot district are mainly affected In Madras no connection can be traced with the hills Possibly here, as elsewhere, the failure lies in taking the district as a unit If smaller units were examined the correlation with certain definite localities and certain definite water supplies would be probably very largely established

4 The outline map of India (Map 9), shows a composite rough picture of the distribution of deaf-mutes in India from the several census reports There are three main points of interest

(1) The maximum intensity is along the Himalayas and in the Naga and Burma hills

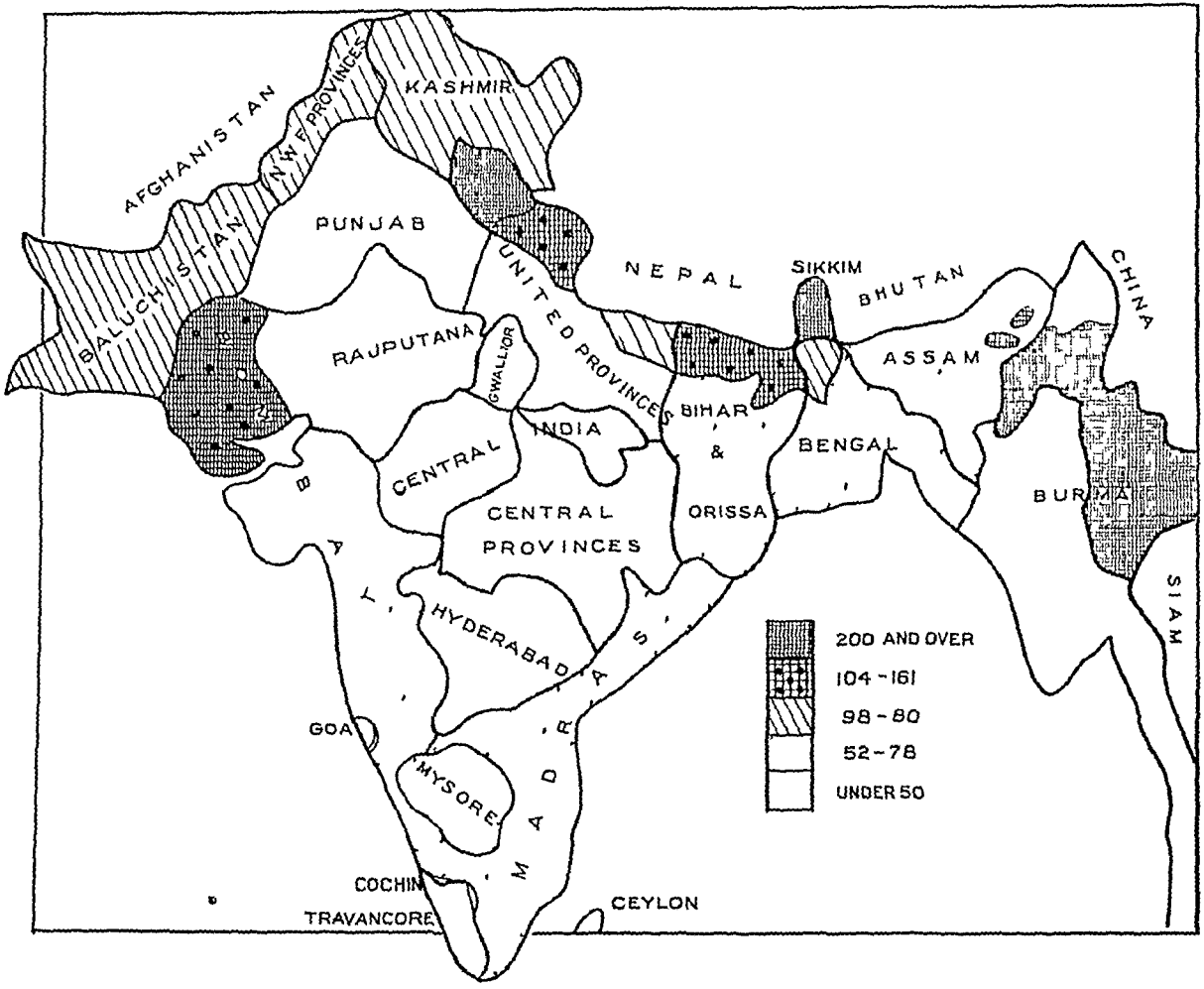
(2) A minor point in distribution lies in those areas bordering the sea board of India, which is of interest as being a nearer a source of sea iodine than the central Indian plateau

(3) The central area of the Peninsula is practically free

5 In a map (Map 10), an attempt has been made to show the distribution of deaf-mutes along the northern frontier of India according to districts and rivers, though as has been shown in the examination of the distribution of goitre in the United Provinces areas far smaller than districts (i e, tehsils at the largest and preferably villages) have to be examined if the cause of goitre is to be localized to its source

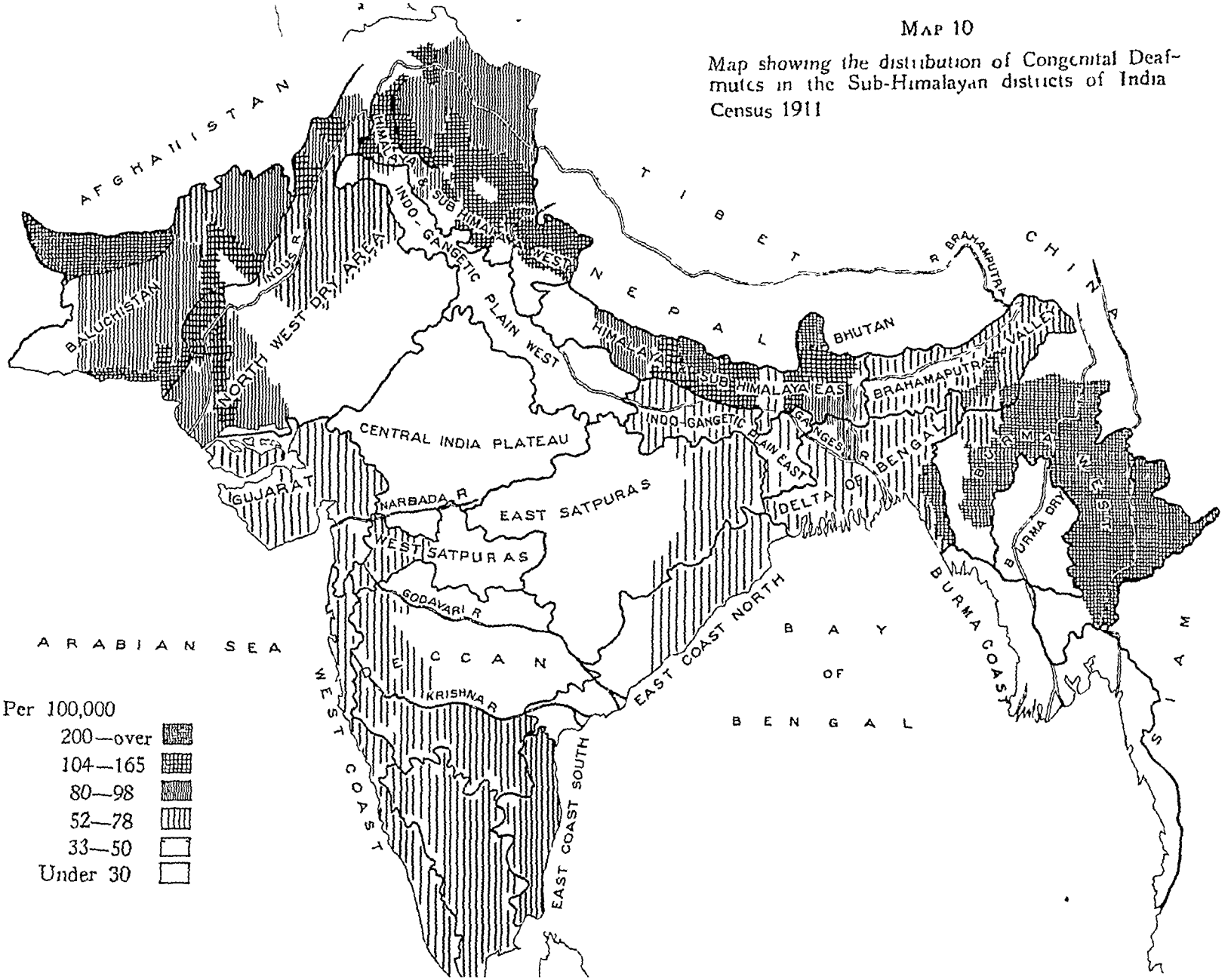
MAP 9

MAP OF INDIA, SHOWING THE APPROXIMATE DISTRIBUTION OF DEAF-MUTES PER 100,000 OF POPULATION



MAP 10

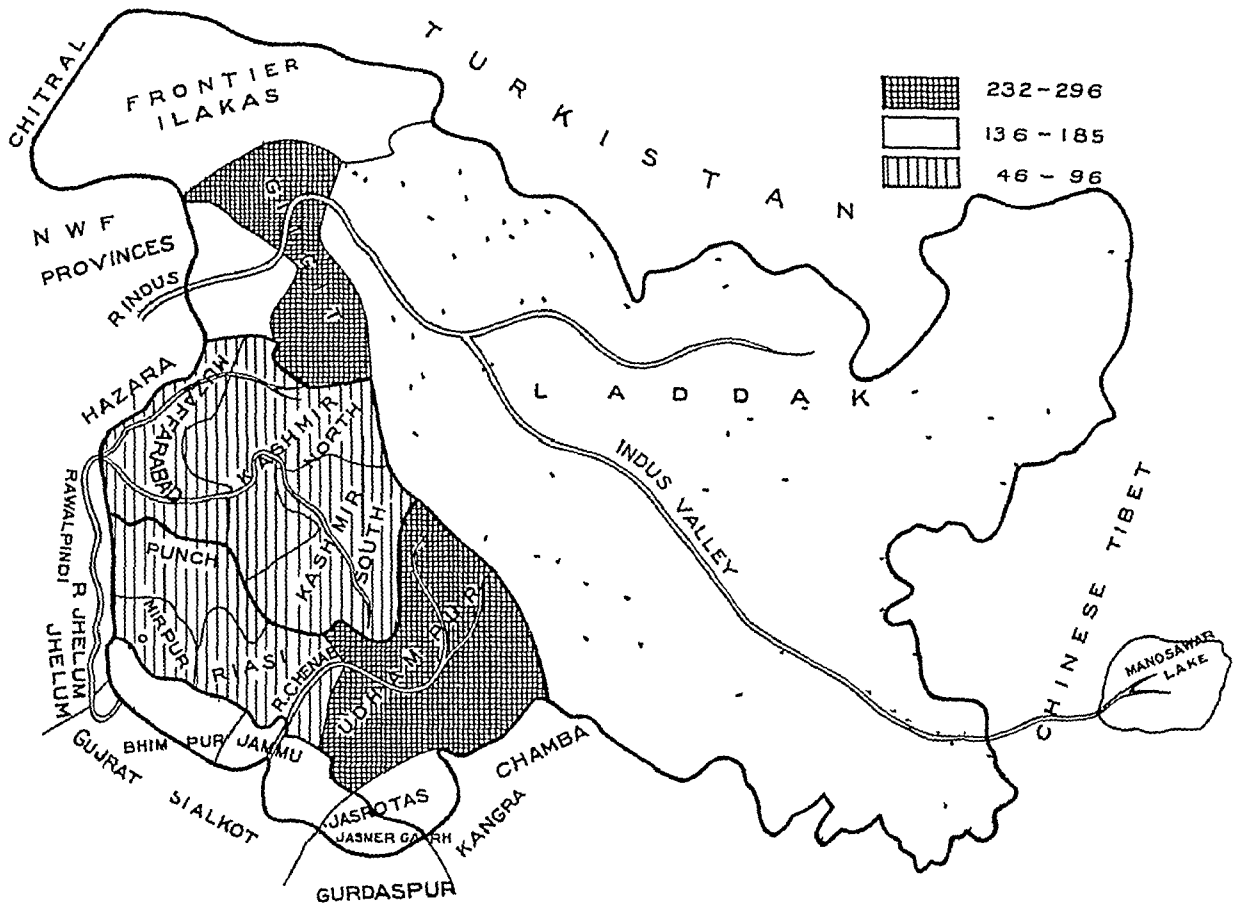
Map showing the distribution of Congenital Deaf-mutes in the Sub-Himalayan districts of India Census 1911



MAP II

MAP OF JAMMU & KASHMIR STATE

SHOWING THE DISTRIBUTION OF CONGENITAL DEAF-MUTES



6 *In the North-West Frontier Province* all four districts situated as they lie at the foot of Afghan hills and bordered by the river Indus are heavily affected Indeed at the 1901 census the only provincial area which showed a higher rate was Kashmir Hazara is a hilly district and a Himalayan tract At the 1911 census the rate per lakh of males was 129 in Dera, 177 in Kohat, 84 in Bannu and 80 in Peshawar

7 *In the Punjab*, the Himalayan area consisting of Chamba, Kangra, Simla, Mandi and Nahan showed the highest rate of 285 The districts of the sub-Himalayan natural division had a rate of 115, all being over 100, except the districts of Gurdaspur, Sialkot and Gujrat which are removed from the higher Himalayas and join the lower hills of Kangra, Jammu and Kashmir An examination of the Punjab sub-Himalayas by tehsils showed that those tehsils which lie close to the hills or abound in moisture give the highest rates Central Punjab and the plain districts are relatively free, the rates lying between 58 and 94 Deaf-mutes are especially found along all the Punjab rivers and are perhaps in greater quantity along the Indus which bounds the North-West Frontier Province than along other rivers

8 The map (Map 11) of *Jammu and Kashmir* shows the rates of deaf-mutes per 100,000 males and females for the different districts of the natural divisions, and is of especial interest since the State is perhaps the most heavily affected in India and is traversed by the valleys of three great rivers of India–the Indus, the Chenab and the Jhelum From south to north, the districts of the natural divisions show the following rates (1911 census) —

TABLE V

Tract		District	Rates per 100,000	
			Male	Female
I Semi-mountainous	1	Jasrota	156	108
	2	Jammu	80	48
	3	Mirpur	171	136
II Outer Hills	1	Udhampur	245	220
	2	Riasi	46	30
	3	Punch	48	43
	4	Mirpur	95	82
III Jhelum Valley	1	Kashmir North	64	49
	2	Kashmir South	98	77
	3	Muzaffarabad	61	54
IV Indus Valley	1	Laddak district	185	165
	2	Gilgit	496	553

9 *The United Provinces* illustrate the usual features, viz, the highest rates in the Himalayan Hill tract and the next highest in the sub-Himalayan tract The importance of tracing each endemic area to its local limits is illustrated in the case of this province, which has been the subject of comparatively detailed examination, as proving the closest possible contact between local endemic areas and specific lime-water supplies

10 *In Bihar and Orissa Province*—The main prevalence is, as usual, in the sub-Himalayan districts or along rivers flowing directly from the Himalayas All endemic areas lie north of the Ganges and are watered by the Himalayan rivers Of all districts Champaran easily preserves its notorious position for being at each census by far the worst affected as may be best indicated in the following table —

TABLE VI

Deaf-mute rates per 100 000 of males

	1921	1911	1901	1891	1881
North Bihar—	107	132	150	210	261
Champaran	236	208	275	131	367
Muzaffarpur	130	144	145	156	225
Saran	105	127	135	195	188
Darbhanga	83	121	117	155	179
Bhagalpur	31	66	127	180	225
Purnea	61	139	130	189	283
South Bihar	41	67	63	91	175
Patna	39	61	61	62	227
Gaya	37	57	49	101	183
Shahabad	67	59	51	136	121
Monghyr	36	86	88	110	172
Orissa	40	72	61	116	126
Cuttack	32	87	89	115	116
Balasore	11	65	86	109	176
Puri	78	51	50	124	95
Chota Nagpur Plateau	43	63	60	65	92

In Champaran, the worst area lies in the south and north-west of the district, which is watered by the Gandak and its tributaries, especially is this so in the Motiharı thana which is bisected by the Burı Gandak and by its tributary the Dhanantı, the latter river having a specially bad reputation for its goitre-producing properties The rate in this thana is 350 per lakh, whilst in Adapur thana the rate falls to 60 per lakh, an interesting example of local variation To ask a man if he comes from this endemic area is equivalent to asking him if he is a complete fool

In the Purnea district, goitre and deaf-mutism are excessively rife along the Kamla river and were noted here as early as 1788 when the author of Riyazu-s-slatin wrote 'Tumours of the throat in men and women as well as in wild beasts and birds are common' In Saran, the area to the north of this district where the Gandak divides it from Champaran is the area of greatest endemicity though even here endemic goitre is far less than on the north bank of the Gandak From the point where the Gandak leaves Champaran its connection with deaf-mutism ceases and the villages on its bank in Muzaffarpur and Saran are comparatively free In Muzaffarpur district, the sadar thana suffers twice as much as any other thana The whole course of the Burı Gandak river in this district and also part of the Bagwatı river lie within this thana

In Darbhanga district, deaf-mutism is most frequent firstly in a thana bounded by two rivers and secondly in those two thanas lying between the Burı Gandak and the Ganges

In Monghyr district, deaf-mutism is more common along the banks of the Burı Gandak than elsewhere in the district

11 *In Bengal province*—Deaf-mutes are by far most common (1) in the hills of Sikkim, (2) Darjeeling, (3) Chittagong Hill Tracts (1921 census) and (4) in the sub-Himalayan district of Jalpaiguri and in Cooch Bihar State than elsewhere As usual the rate is highest in these districts through which the Himalayan drainage passes on its way to the sea Those districts on the sea face of the delta are comparatively free

12 *In Assam province*—Assam consists of two valleys, the Brahmaputra valley and the Surma valley surrounded by hills Both valleys are alluvial and the Brahmaputra valley is the endemic home of kala-azar There are two endemic foci of deaf-mutism, firstly in the Naga Hills, where the disease is extraordinarily prevalent, being some seven times the provincial ratio The second focus is in the Lushai Hills The other hills and districts are comparatively free, averaging 70–90 cases per lakh of population

13 *In Burma*—There is a fringe of districts with a high ratio on the north and east side of the province There is little goitre in the valleys, the deformity being localized in each district to the hills, that is to 7 per cent of the population Deaf-mutes and cretins are rarely seen without goitres Two causative factors are recognized (1) mountains and (2) something in the

drinking water which in the hills is obtained from small streams In the North Shan States this series of diseases may be traced to particular streams

Amongst the Kachins who inhabit the valleys and steep hill-sides of the north and north-east frontiers, the rate per lakh reaches the enormous figure of 1,004 It is their custom to eat a vast amount of lime in powdered form which may be an important contributory factor The other racial rates work out at Kadu 295, Chin 252, Shan 150, Burmese 50 and Arakanese 29

The following table sets out the number of deaf-mutes according to natural divisions (1911) census per 100,000 population —

1	Central Basin	45
2	Deltaic Plains	33
3	Northern Hill districts	234
4	Coast ranges	43
5	Specially administered	209

The maximum prevalence according to districts were (1) Bhamo 474, (2) Myitkyina 474, (3) Chin Hills 401, (4) North Shan States 350 The maximum area of intensity was said to be at Shwega in Kachin Hills where the rate was reported as 7,000 per lakh of population

14 *In the Central Provinces*—No endemic area appears to have been recognized but deaf-mutes are reported most in the valley of the Nerbudda river It is suggested the afflicted congregate at the ghats and fairs which are frequent along its course

15 *Madras Province*—The deaf-mute figures are small and are highest in North Arcot, where they reach 112 per lakh

16 *Bombay Presidency*—The distribution shows the greatest incidence round the mouth of the Indus in Sind, especially in Hyderabad Sind where the figures at the last five censuses terminating with 1921 have been, 120, 136, 51, 143 and 100 per lakh There is a slightly increased figure for the Konkan district

Summary

1 In India as a whole, congenital deaf-mutes, cretins and goitrous persons are located in a main endemic area in the Himalayas and in those districts bordering the Himalayan foot-hills, especially in those areas where the drainage water is carried from the Himalayas to the sea

2 Around the Indian sea-coasts the rates for deaf-mutes are higher than in the Central Indian Provinces and States

3 Within the several provinces the congenital deaf-mute rate varies according to locality in a most remarkable degree Examples are given

4 Where the local distribution of this disease group has been investigated it is associated with a definite water-supply and in that water-supply lime is usually found present in excessive amounts

5 A map of the districts along the northern boundary of India with their relation to the river drainage system is given and outstanding features of each province so far as these are known are briefly commented on

6 Nowhere in India or Burma is the deaf-mute rate higher than amongst the Kachins of North Burma where it reaches the enormous figure of 1,004 per lakh of population These Kachins drink water from hill-streams which are no doubt impregnated with calcium and moreover it is customary amongst the Kachins to eat calcium as a powder in large quantity

7 In the study of this group of diseases there is much investigation waiting to be undertaken by active research workers in the many severe endemic centres

8 Maps showing the rough distribution of goitre in India have been prepared by McCarrison (*Ind Jour Med Res*, January 1915) and by Megaw (*Ind Med Gaz*, June 1927) and may be consulted with advantage

Explanation of Plate LVII

Fig 1 A Burmese Cretin

Note (1) The bones (except skull) have failed to develop

(2) The trunk though small compared with the head appears massive against a background of diminutive limbs Abdomen large and flabby

(3) Skin bloated and swollen Hair thin and falls out

(4) Nose depressed and pig-like, lips thick Eyes concealed by thick eyelids

(5) Hands and feet broad, poddy and floppy

(6) Legs covered with rolls of fat

„ 2 A group of thin cretins in Padrauna

Average age, over 20 Height, about 4 feet Fasting blood-sugar, low Sugar tolerance, high

PLATE LVII

Fig 1

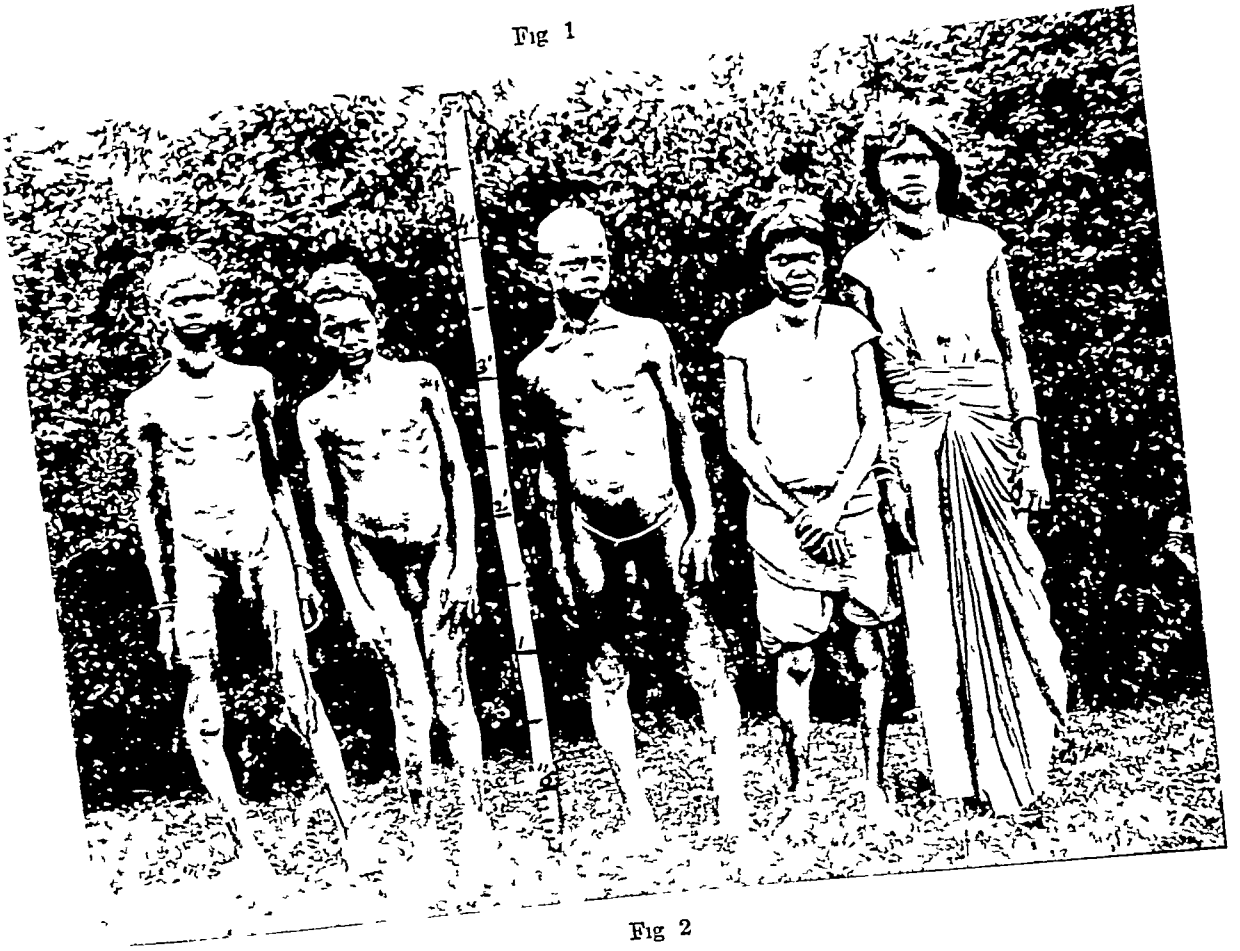


Fig 2

EFFECT OF OPIUM ON THE BLOOD-SUGAR OF NON-DIABETICS

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IN a previous paper (1930) we gave an account of the administration of small doses of opium on blood-sugar of diabetic patients In this paper we propose to give the results of our observations on the administration of small and large doses of opium on the blood-sugar of more or less normal individuals It was our endeavour to get absolutely normal and healthy Indians, but we found it very difficult to induce them to come and lie up in hospital, which condition was absolutely necessary to keep the patients under very careful observation and absolute control We succeeded in getting a few of these individuals but had also to include cases suffering from mild skin affections, dyspepsia, etc, which, for all practical purposes, may be classed as normal inasmuch as they suffered from no disturbance of carbohydrate metabolism as is the case with diabetics

Opium was administered in the form of a mixture so constituted that one ounce contained 1 grain of the drug The taste and smell of opium was effectively concealed by putting in oil of citronella so that the patient had no idea as to the nature of the drug he was taking This was done to exclude the psychic element The dose to commence with was 1 or 2 grains daily and was increased to 14 grains in a short time without the least discomfort to the patient In some cases the dose was gradually reduced to nothing but in others the drug was suddenly stopped without producing any subjective or

objective symptoms It may be noted here that most of the patients left the hospital with the idea that the drug which was being given to them was an excellent general tonic

The description of cases given below should be read with the details given under each case The quantity of urine passed, blood-sugar content, body-weight and the amount of opium administered are all recorded in the vertical columns

Case 1—N S, a Hindu male aged 10 Wassermann reaction of blood strongly positive, blood-sugar was found to be abnormally low His only complaint was that he felt weak He was put on small doses of opium commencing with 1 grain daily which was increased to 2 grains daily and later on to 3 grains daily He felt stronger and his blood-sugar also

Case 1

Days of treatment	Quantity of urine	Blood-sugar per cent	Body-weight in lb	Opium administered daily in grains
1		0·045		1
2			76	1
3				2
4				2
5	1,020			2
6				2
7	660			2
8	600	0·054		3
9	1,020			
10	780		78	
11				3
12	1,140	0·070		Discontinued
13				
14				
15				
16				
17				
18				
19				
20				

went up definitely though it was still below normal As this patient did not want to stay in hospital, the course of opium treatment could not be continued and he was discharged The blood-sugar content showed some improvement The patient got a total dose of 24 grains in 11 days, the maximum dose being 3 grains daily

Case 2—G D, a Hindu male, aged 35 years, admitted into hospital on 13-1-29 suffering from gastric trouble He complained of pain and discomfort in the epigastric region, anorexia, nausea and sometimes vomiting He was put on small doses of opium commencing with 1 grain daily, later increased to 2, 3 and 4 grains daily The pain disappeared on the 4th day with 2 grains daily doses and the patient felt very comfortable The patient was given a total dose of 31 grains in 12 days the maximum dose being 4 grains daily Blood-sugar content was not affected

CASE 2

Days of treatment	Quantity of urine	Blood-sugar per cent	Body-weight in lb	Opium administered daily in grains
1	450	0·081		1
2	1,080			1
3	600			2
4	540			2
5	600		100½	2
6	300			2
7	720			3
8	480	0·090		3
9	540			3
10	660		101	4
11	840			4
12	660			4
13				Discontinued
14				
15				
16				
17				
18				
19				
20				

Case 3—S R, a Hindu male, aged 27 years No complaint except a small ulcer on the sole of the foot The patient was put on 1 grain of opium daily which was later increased to 2, 3 and 4 grains daily The patient felt a sense of well-being and was more cheerful when the drug was being administered The blood-sugar and the quantity of urine remained unaffected This patient was given a total dose of 13 grains in 18 days, the maximum dose being 4 grains daily

CASE 3

Days of treatment	Quantity of urine	Blood-sugar per cent	Body-weight in lb	Opium administered daily in grains
1				1
2				1
3	1,800	0·06S	105	2
4	1,860			2
5	2,220			2
6	1,980	0·075		2
7	2,460			2
8	1,560			2
9				2
10	1,800			4
11	1,440			4
12	1,740			3
13	1,800	0·06S		3
14	1,920			3
15	1,140			3
16				3
17	1,440			2
18	1,920			2
19	1,350			
20				

Case 4—Y, a Mohammedan male, aged 23 years, a normal and healthy individual in every way He was put on opium commencing with 1 grain increased to 2, 3 and 4 grains daily He admitted improvement in his general health, had a general feeling of well-being

and gained weight due to the liberal hospital diet The patient had a total amount of 43 grains of opium in 17 days The blood-sugar did not show any changes under the effect of the drug

CASE 4

Days of treatment	Quantity of urine	Blood-sugar per cent	Body-weight in lb	Opium administered daily in grains
1		0 09		1
2			100	2
3				2
4	1,260			2
5	660			3
6	660	0·084		3
7	1,920			3
8	1,380			3
9				3
10	1,140		110½	4
11	1,380			4
12	1,380			3
13	1,440	0·082		3
14	1 680		110½	2
15	1,740			2
16				2
17	1,260		108	1
18	780			Discharged
19				
20				

Case 5—Y, a Mohammedan male, aged 36 years He was a normal and healthy individual He was put on 1 grain of opium at first, later increased to 2, 3 and 4 grains daily He felt that his health was generally improved after the course of treatment and he was more vigorous The blood-sugar remained unaffected, the quantity of urine was not affected

Case 5

Days of treatment	Quantity of urine	Blood-sugar per cent	Body-weight in lb	Opium administered daily in grains
1		0·077		1
2				1
3			95	2
4				2
5	840			2
6				2
7	840			2
8	840			2
9	1,260			2
10	720		95	2
11				2
12	900			4
13	720			4
14	960			4
15	780	0·082		4
16	900			2
17	1,020			2
18				2
19	900			2
20	600			Discontinued

Case 6—S C, a Hindu male, was admitted into hospital for loss of weight and strength for no apparent cause The patient was put on 1 grain of opium daily which was increased on the second day to 2 grains daily and later to 3 and 4 grains daily The patient did not seem to derive much benefit from the use of the drug The loss of weight continued steadily and there was a definite reduction in the blood-sugar The quantity of urine passed showed a tendency to increase The patient received a total of 45 grains in 16 days, the maximum dose being 4 grains daily

CASE 6

Days of treatment	Quantity of urine	Blood-sugar per cent	Body-weight in lb	Opium administered daily in grains
1	660	0·096		1
2	420			2
3	360		81	2
4				2
5	420			3
6	720			3
7	1,440		80½	4
8	780	0·052		4
9	1,320			4
10	1,020		73½	4
11				4
12	1,260			3
13	2,100			3
14	2,280			2
15		0·050		2
16	1,140		67½	2
17				Discontinued
18				
19				
20				

Case 7.—P K B, a Hindu male, aged 24 years, admitted into hospital on 4-11-29. He suffered from mild attacks of lymphangitis every now and again but is perfectly free from it now. He was put on 2 grains of opium daily, later increased to 2, 4 and 5 grains daily. The patient improved very much in general health during the course of opium administration and his body weight showed a slight increase. The blood-sugar was not affected in the slightest degree. The urine showed a distinct tendency to increase during the administration. The patient had a total of 49 grains in 13 days, the maximum dose being 5 grains daily.

Case 7

Days of treatment	Quantity of urine	Blood-sugar per cent	Body-weight in lb	Opium administered daily in grains
1			95½	
2	1,420	0 106		2
3	1,200			2
4	2,760			3
5	2,460		97	3
6	1,980		..	4
7	2,460		..	4
8	2,400		.	5
9	2,340	0 108	.	5
10	2,340		.	5
11	1,920			5
12	2,160		98¾	5
13	2,040			3
14	2,400			3
15	2 280			Discontinued
16				
17				
18				
19				
20				

Case 8—M S, a Hindu male, admitted into hospital for general debility and loss of weight He was put on opium commencing with 1 grain daily, later increased to 2, 3 and 4 grains daily The patient felt a general sense of well-being but his weight remained unaffected for the period of 3 weeks he remained in the hospital There was a marked improvement in the general condition, the appetite improved and the patient thought he was getting better There was practically no change in the blood-sugar content, the quantity of urine showed marked variations during treatment but was above the normal The patient had a total dose of 58 grains in 23 days, the maximum dose being 4 grains

CASE 8

Days of treatment	Quantity of urine	Blood-sugar per cent	Body-weight in lb	Opium administered daily in grains
1	0·072			—1
2				1
3				2
4				2
5	1,928		116	2
6				2
7	1,740	0·081		3
8				3
9				
10	2,700			3
11				3
12	2,400		115	4
13	1,740			4
14	1,140			4
15	430	0·077		4
16	1,440			4
17	2,160			4
18				4
19	2,100		114	1
20	1,980			1
	2,700			1
	2,220	0·075		1
	2,520		114½	1

Case 9—B M, a Hindu male, aged 30 years, admitted into hospital on 7-2-29 with mitral stenosis He was put on 2 grains of opium daily, later increased to 3, 4, 6 and 7 grains daily The patient's general condition showed remarkable improvement, in all probability due to rest and good diet of the hospital The weight showed a definite increase and the patient felt remarkably well The only distressing feature was constipation which had to be relieved by saline aperients The blood-sugar showed no changes The quantity of

urine passed showed marked variations, but it could not be said that it either increased or decreased even with such doses as 7 grains daily The patient was given a total dose of 65 grains in 16 days, the maximum dose being 7 grains daily

CASE 9

Days of treatment	Quantity of urine	Blood-sugar per cent	Body-weight in lb	Opium administered daily in grains
1	600		103½	
2	1,140			2
3	900	0 120		2
4	2,540			2
5	480		107½	2
6	780			2
7	600			3
8	480			3
9	600			3
10	720			4
11	480			4
12	840		110¾	Discontinued
13				Do
14	1,080			Do
15	540			Do
16	840			Do
17	600	0·092		6
18	840			6
19	900			6
20	1,080			6
	840		112	7
	420			7
	480			Discontinued

Case 10—A A M, a Mohammedan male, aged 21 years, admitted into hospital on 11-6-28 suffering from affections of the skin He appeared to be normal in every other respect Commencing with 1 grain of opium daily, the quantity was increased to 7½ grains

daily The patient felt much brighter and improved in general health, there was a slight increase in weight The quantity of urine passed showed fluctuations but on the whole showed a definite increase The patient was given a total dose of 102 grains in 22 days, the maximum dose being 7½ grains daily

CASE 10

Days of treatment	Quantity of urine	Blood-sugar per cent	Body-weight in lb	Opium administered daily in grains
1			85½	
2	1,005	0·085		1
3	1,015			1
4	960			1
5	1,030			1
6	1,020			2
7	1,030			2
8	1,100		85	3
9		0·08		3
10	1,170			4½
11	1,620			4½
12	1,200			4½
13				4½
14	1,560			6
15	1,560		86¼	6
16	1,680	0·08		6
17	1,680	0·081		6
18	1,560			6
19	1,920			7½
20	1,800			7½
	1,620			7½
	1,980			7½
	1,680			7½

Case 11—A K G, a Hindu male, aged 26 years, was admitted into hospital on 16-1-30 for irregular fever Commencing with 2 grains of opium daily the quantity was gradually

increased to 12 grains daily The patient felt quite comfortable in spite of the large doses of opium he was receiving His weight showed an increase and the blood-sugar showed a decrease, especially, when such large doses as 12 grains daily were administered No ill effects were observed when the maximum doses of opium suddenly discontinued The patient received altogether 117 grains in 17 days, the maximum dose being 12 grains daily

CASE 11

Days of treatment	Quantity of urine	Blood-sugar per cent	Body-weight in lb	Opium administered daily in grains
1		0·126	108½	2
2				2
3				3
4				4
5				4
6			110½	4
7				6
8				6
9				6
10		0·10		8
11				8
12				8
13				8
14				12
15				12
16				12
17		0·085		12
18		..	.	Discontinued
19			.	
20				..

Case 12—N D, a Hindu male, aged 40 years, admitted into hospital on 16-11-29 for blood and mucus in stool with griping Previous history of dysentery, two years ago After a total dose of 8 grains of opium in 4 days, the patient complained of headache and giddiness but his pupils remained perfectly normal The dose was, therefore, gradually increased to 16 grains daily With larger doses the symptoms at first complained of disappeared and he felt very comfortable The stool became much less frequent and the griping entirely disappeared The patient, however, suffered from constipation and the

bowels had to be moved daily with saline purgative The blood-sugar remained unaltered even with large doses The quantity of urine did not appear to alter much, but with doses of 14 grains, showed a tendency to decrease The patient got a maximum dose of 16 grains of opium daily with absolutely no untoward result He felt very bright and cheerful The opium was then suddenly stopped, but no untoward symptom, subjective or objective, was observed The patient was given a total dose of 188 grains in 23 days, the maximum dose being 16 grains daily

CASE 12

Days of treatment	Quantity of urine	Blood-sugar per cent	Body-weight in lb	Opium administered daily in grains
1			96	2
2	2,100	0·105		2
3	1,200			2
4	2,580		94¾	
5	2,490			3
6	2,460			3
7	1,940			5
8	2,400	0·100		5
9	3,240			6
10	2,340			6
11	2,840	97		6
12	2,400			8
13	2,460			8
14	2,840			10
15		0·092		10
16	1,580			12
17	2,400			12
18	2,400		97¾	12
19	2,400			14
20	2,580			14

DISCUSSION OF RESULTS

Effect of opium on general health—Almost all the patients declared that they felt much brighter and comfortable and there was a sense of general well-being In Case No 2 admitted for epigastric pain and discomfort the pain

entirely disappeared and the patient regained his appetite and his general health improved Among the two cases (Nos 6 and 7), admitted for loss of weight without any demonstrable cause, one did not improve at all, the loss of weight continuing during the whole course of treatment In the other, however, though there was a slight loss of weight (1½ lb in 23 days), the rate of loss of weight was much less than what it was before he was put on opium treatment and the patient felt generally better and the weakness became much less One case (No 11) complained of giddiness and headache after he had a total dose of 8 grains of opium but as there were no signs or symptoms of overdose of opium present the dose was increased Curiously enough, all his symptoms disappeared The patient felt very much better with such doses as 10 to 16 grains daily This patient had a total of 188 grains of opium in 23 days, the maximum daily dose being 16 grains It is worthy of note that the drug in several cases was discontinued suddenly the patient being none the worse for it

Effect on the urinary output—Most of the patients showed an increase in the total urinary output varying from slight to a marked degree In 4 cases, the urinary output was unaffected by the drug In no case we found a decrease in the total daily output of urine

Effect on blood-sugar—In most of the cases the blood-sugar level remained constant In 4 cases (Nos 6, 8, 10 and 11), there was a slight decrease, and in 3 cases (Nos 1, 2 and 5) the blood-sugar content was slightly increased In case No 1, where the blood-sugar was found to be abnormally low in the beginning of treatment, much benefit followed the course of opium treatment The blood-sugar increased and tended to approach the normal figure The weakness became much less This point needs more investigation

Effect on body-weight—Excepting the two cases mentioned before, there was more or less increase in the body-weight in all the cases varying from slight to moderate degree Almost all the cases complained of constipation in a slight or severe form

Summary and conclusions

(1) Small doses of opium such as 1 to 3 or 4 grains daily produced a sense of well-being especially in those individuals who were suffering from minor ailments Although the patients were entirely ignorant of the drug they were taking, they all thought it was doing them good and was a tonic The hospital regimen and dietary had undoubtedly a good deal to do with the general improvement in the condition of the patients

(2) The secretion of urine was, if anything, improved In no case was there a decrease in the quantity of urine passed even after such large doses as 10 to 16 grains daily

(3) There was little or no effect on the blood-sugar of normal individuals with no disturbance of carbohydrate metabolism In one case, where the blood-sugar contents were abnormally low, a definite rise was observed

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THE ACTION OF THE VENOM OF THE INDIAN COBRA (*N NAIA VEL TRIPUDIANS*) ON CERTAIN PROTOZOA

BY

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INTRODUCTION

SOLLMANN (1927) considered that unicellular organisms such as infusoria, bacteria and plants were not affected by any snake venom and some workers have agreed with his conclusions It has been stated that venom is toxic to metazoa and complex cellular animal life above the hydra, but there appears to be no experimental evidence in support of this view Whilst working on the action of the venom of Indian cobra *N Naia vel tripudians* we carried out a series of experiments regarding its effect on certain protozoa and this paper contains the results of our observations We tried the effect of the venom on *Paramecium caudatum* as well as some of the other common protozoa such as *Bodo caudatus, Trichomonas hominis, E histolytica* and the ordinary fresh water amœba *E limax* The effect of different concentrations of the venom on these organisms was tested and the changes produced in their movements, their resistance to the poison, the exposure necessary to produce death were studied Finally the effect of change of hydrogen-ion concentration on the toxicity of cobra venom towards *Paramecium caudatum* was worked out in detail

The cobra venom used for this work was kindly supplied to us by the Director of the Haffkine Institute, Parel, Bombay It is a light yellow scale-like substance which dissolves in distilled water forming an emulsion solution

faintly opalescent The coarser particles of this emulsion solution have a tendency to settle to the bottom in the form of a fine flocculent precipitate and therefore the solution has to be shaken repeatedly to get an uniform emulsion Cobra venom is readily soluble in normal saline, but since saline solutions are very toxic to *P caudatum,* an aqueous solution had to be used for these experiments A freshly prepared 1 in 500 solution of cobra venom in distilled water was first made and from this the various concentrations were diluted with ordinary tap-water as these solutions kept the *P caudatum* more or less under natural conditions Dilutions commencing with 1 in 1,000 up to 1 in 15,000 were made by taking in a capillary pipette an equal volume of the venom solution with an equal volume of paramecium culture The mixture was then put in a hollow ground slide and the effects of the venom observed under a low power microscope In this way the effect of different dilutions from 1–1,000 to 1–30 000 could be studied

The behaviour of P caudatum *towards cobra venom*

P caudatum is a common unicellular ciliate found in stagnant ponds and it can be easily grown in the laboratory on hay infusions Good cultures can be obtained in 7 to 10 days Under the low power, these ciliates are seen as rapidly moving organisms, slipper-like in appearance, varying between 120 to 325 μ in size They show peculiar and interesting movements, when they come in contact with any substances which are harmful to them Their behaviour can be best studied on a hollow ground slide in which the paramecium culture is put and a drop of cobra venom solution (1–1,000) is gently added on one side They move towards the harmful substance, and when they come in close proximity to it, they appear to come to a standstill as if not pleased to find themselves in an unpleasant environment They stop for a moment, seem to move forwards and backwards as if undecided and in hesitation, then all of a sudden turn round, move backwards or go off at an angle This behaviour of the paramecium is known as 'avoiding reaction' and has been fully discussed by Jennings and was noticed by Acton in case of the cinchona alkaloids

To study the action of different dilutions of cobra venom we improvised slides having five or six small glass cells built on it This can be done by fixing on an ordinary slide small glass rings 1 c mm in depth cut from a piece of soft glass tubing, and fixed with collodion or melted paraffin wax These cells have roughly a capacity of 1 c c and the effect of cobra venom in different dilutions could be studied on these ciliates by simply moving the slide from one cell to the other under the low power of microscope Evaporation was prevented by putting a cover slip over the rings One of these chambers was always kept as control, containing equal volume of the culture and tap-water and the changes in movements and the death interval was contrasted side by side with those exposed to the venom

Strong solutions of the venom appeared to be very toxic to these ciliates Concentrations of 1 in 1,000 or 1 in 2,000 immediately paralyse the movements of the paramecium and they at once die and sink to the bottom The body of the ciliates swell up, burst and completely disappear With weaker dilutions such as 1 in 4,000 to 1 in 8,000 the ciliates at first become more active on coming in contact with the cobra venom, and soon their movements become irregular and incoordinated They appear to be moving aimlessly in all directions as if trying to avoid the unsatisfactory surrounding and eventually collect at the periphery of the glass well which probably is a safer area owing to lower concentrations of the venom The corkscrew-like movements become very marked and the individuals soon appear to lose power of translation movement and settle down to the bottom of the chamber and move by crawling only At first they can be made to swim on agitating the slide, but later become paralysed and finally die

Weaker concentrations such as 1 in 15,000 to 1 in 30,000 do not appear to have any marked effect on these ciliates at first After about half an hour, they begin to show corkscrew movements and a tendency to sink to the bottom of the chamber and aggregate in large clumps chiefly at the periphery It appears as if they are in uncongenial surroundings and want rest From time to time individuals who had been resting detach themselves from the main clump and move away while others come and take their place Individual ciliates also settle down at the bottom as if to rest a while and then move away again

Table I gives the effect produced by different dilutions of the venom —

TABLE I

Number	Final dilutions of cobra venom	Death in minutes	Remarks
1	Control	No effect	All quite active and alive after two hours
2	1 in 1,000	Immediate	The organisms become immediately paralysed and die settling down to the bottom of the well
3	1 in 2,000	5–6	Rapid movements at first followed by slowing, paralysis and death All organisms were killed and the protoplasm of the cell became opaque
4	1 in 4,000	12·0	
5	1 in 6,000	15 0	
6	1 in 8,000	16 0	
7	1 in 10,000	18·0	Corkscrew-like movements at first, finally paralysis and death A few organisms showed resistance and did not die
8	1 in 15,000		Do
9	1 in 20,000	30–40	Movements become slow, tendency to aggregate in colonies Majority die but some remained active for nearly two hours
10	1 in 30,000	45–60	Many of the ciliates died after 40 to 60 minutes but some remained active
11	1 in 40,000	120 0	Little or no effect

A perusal of Table I shows that up to 1 in 8,000 the venom has a very toxic action, but with weaker dilutions up to 1 in 30,000 the paramecia take a longer time to die, death taking place in ½ to 1 hour. Some individuals were not killed even after an exposure for two hours. Dilutions over 1 in 30,000 had no effect.

It is interesting to note that there are certain individuals in these communities who do not conform to the general rule and showed a greater resistance to the action of the venom. They move about for a longer time and do not die even in double the time. Acton (1921) made some very interesting observations about the individual variations regarding the resistance of these ciliates and showed that these variations depended on race, individual resistance, hydrogen-ion concentration and the age of the culture. The older cultures were more resistant than the younger cultures. When a culture was allowed to grow for 2 or 3 months these ciliates reacted irregularly to drugs. Cultures 7 to 10 days old gave fairly constant reaction so far as drugs were concerned.

Effect of change of pH

It has been shown that the toxicity of drugs towards paramecium is altered by changing the hydrogen-ion concentration of the substrate. Acton (1921) observed that whereas the minimum lethal concentration of quinine to paramecium at a pH of 7 was 1 in 10,000, at a pH of 8 it was 1 in 70,000. It was, therefore, thought desirable to see how a change in the pH of the culture would affect the toxicity of cobra venom towards *P. caudatum*.

It has been suggested that variations in the toxicity of drugs to paramecium were due to the amount of CO_2 present in the culture media and it is advised that these ciliates should only be tested after bubbling air through the tube containing their culture. The presence of the amount of CO_2 thus produces considerable variations in the hydrogen-ion concentration of the medium. The pH value of the stock culture varies between 6·9 and 7·2. These small variations are due to the age of the culture, the fresh culture being more alkaline as compared to the older ones. The pH of the culture was altered on the alkaline side by adding N/10 NaOH solution and lowered by the addition of weak acids. To change it on the acid side hydrochloric acid could not be used as it killed the protozoa immediately. We, therefore, used weak acids like carbonic acid by bubbling this gas through the medium, the required hydrogen-ion concentration could be obtained within certain limits. It was found difficult to lower the pH value to less than 6 since the solutions become fully saturated with CO_2 and further solution of the gas becomes very difficult. By using acid phosphates the pH could be further reduced, but this meant the addition of large quantities of this weak acid solution which diluted the *Paramecium caudatum* culture so much that very few organisms could be seen when the pH value of the substrate was reduced below 6·5.

A perusal of Tables II, III and IV will show that the pH of the substrate has a very important effect on the resistance of the paramecium against cobra

TABLE II

Number	Concentration of cobra venom	DEATH INTERVAL WHEN pH OF CULTURE WAS ALTERED WITH N/10 NaOH	
		pH 8·0	pH 7·0
1	1 in 2 000	5 minutes	44 minutes
2	1 in 4 000	18 „	15 „
3	1 in 6 000	20 „	40 „
4	1 in 8,000	22 „	48 „
5	1 in 10 000	40	60 „
6	1 in 15 000	60 ,	Over 2 hours
7	1 in 20,000	Over 60 ,	Do
8	1 in 30 000	Over 120 „	Do

TABLE III

Behaviour of paramecium towards cobra venom at different pH values as altered by passing CO_2 gas

Number	Concentration of cobra venom	DEATH INTERVAL WHEN pH IS ALTERED WITH CO			
		7	6·5	6·0	5·8
1	1 in 2,000	4·8	3·2	3	2
2	1 in 4,000	15	12	12	5
3	1 in 6,000	40	30	30	15
4	1 in 8,000	60	40	30	30
5	1 in 10,000	60	60	45	35
6	1 in 15,000	120	60	50	45
7	1 in 20,000	120	60	60	60
8	1 in 30,000	120	150	60	60

venom If we take the death interval of paramecium at pH 7 as standard, it will be seen that on lowering the pH below 7, the protozoa die rapidly even

TABLE IV.

Behaviour of paramecium towards cobra venom at different pH values as altered by diluting with phosphate buffer of a definite pH

Number	Concentration of cobra venom	DEATH INTERVAL WHEN pH IS ALTERED WITH N/10 $NaHPO_4$				
		8·6	8	7·5	7	6·5
1	1 in 2,000	16	16	12	7	5
2	1 in 4,000	30	30	25	18	15
3	1 in 6,000	50	45	40	28	25
4	1 in 8,000	53	50	50	45	35
5	1 in 10,000	60	60	60	50	40
6	1 in 15,000	60	60	60	60	50
7	1 in 20,000	120	120	120	120	55
8	1 in 30,000	120	120	120	120	60

in very weak concentrations of the venom, on the other hand if the pH is raised they become more resistant and in weak concentrations of the venom seem only to be paralysed for a short time and have a tendency to aggregate in colonies The explanation of this phenomenon will be discussed later

The cellular changes in P caudatum *killed by cobra venom*

Three-fourth c c of a fresh culture was put in each of the chambers In each of these slides an equal volume of cobra venom solution of the following concentrations, 1 in 1,000, 1 in 2,000, 1 in 8,000 and 1 in 10,000, were added and thoroughly mixed After 5 minutes smears from the control and the above mixtures were taken, fixed and stained after the Heidenhain's method after wet fixation by Schaudinn When stained in this way and the slides were examined under the high power of the microscope, important structural changes were found to have occurred in the body of the ciliate The normal *Paramecium caudatum* appears quite clear, the ecto- and endo-plasm can be distinguished when stained by the Heidenhain's Iron Hæmotoxylin method The protoplasm appears granular in nature, the macro- and micro-nuclei appear clear and compact With the opal blue method of staining, the cilia and their trichocytes can be clearly demonstrated The vacuoles are distinct and clear

After exposure of the paramecium to 1 in 1,000 and 1 in 2,000 cobra venom solution the protozoa appear to swell up and the general appearance becomes hazy, differentiation can no longer be made between ecto- and endo-plasm, the cilia mostly appear to have been cast off, the vacuoles become enlarged

but remain clear, the whole protoplasm appears to be vacuolated The macro-nucleus appears to be swollen and undergoing karyorrhexis, the micro-nucleus cannot be differentiated The chromatin becomes very indistinct and is found in form of granules in the protoplasm Achromatic changes are also noticeable The cilia become detached and many of them are destroyed The body of the organism denuded of cilia looks swollen and engorged The organism in fact looks like a mass of spongioplasm (*see* Plates LVIII and LIX)

Other protozoal organisms

Bodo caudatus—These protozoa were found to be very resistant to the toxic effects of cobra venom Under a high magnification they seem to be rapidly moving across the field and do not appear to be affected very much by low concentrations of the venom In dilutions of 1 in 2,000 and more they could survive an hour or so but with concentrations as high as 1 per cent their death is very rapid The following table shows the death interval with different concentrations of the cobra venom

TABLE V

Final dilution	Death interval	Final dilution	Death interval
1 in 200	5 minutes	1 in 1,000	20–30 minutes
1 in 400	10–15 minutes	1 in 4,000	About 40 minutes
1 in 600	15 minutes	1 in 8,000	More than 60 minutes
1 in 800	20 minutes	1 in 10 000	More than 120 minutes

Trichomonas hominis—These organisms are also very resistant and appear only to be affected by concentration varying from 1–100 to 1–1,000 Trichomonas could survive for 10 to 15 minutes in dilutions of 1 in 400 and between 20 to 30 minutes in 1 in 600 and for over an hour in 1 in 1,000

Entamœba limax and *Entamœba histolytica*—Immediately after mixing with a drop of 1 in 1,000 solution these actively moving amœbæ were seen to become rounded and granular The pseudopodia were not protruded and the amœba were stationary Although the granular protoplasm and the vacuoles were still seen to be moving inside the cell, there was complete paralysis in 40 to 60 minutes The death interval was not always the same in case of the entamœba but it was noticed that they could survive an exposure of 45–60 minutes in a 1 in 2,000 solution of the venom

Microfilaria bancrofti—The microfilaria were actively moving and wriggling about in a drop of warm normal saline A drop of 1 in 1,000 solution of venom was added In about 10 minutes the microfilaria were seen lashing about with greater activity and in about 15 minutes the movements became slower and finally they were paralysed after 30 minutes, the sheath appearing

to have been constructed over the body of the filaria so as to give it a bead-like appearance After 35 to 40 minutes the microfilaria are motionless and appear to have died

Discussion.

The experiments described above show that cobra venom at first somewhat stimulates, then paralyses and finally kills the *P caudatum* Dilutions up to 1 in 8,000 are rapid in their effect, dilutions above 1 in 10,000 paralyse movements and kill after a longer exposure As regards the action of the venom at different hydrogen-ion concentration, it was found that if the pH of the substrate is on the acid side the venom becomes more toxic and if it is increased on the alkaline side the toxicity of the venom is decreased This action is opposite to what was found in case of the cinchona alkaloids The explanation of this phenomenon is not quite clear It is possible that the action is of the nature of an enzyme which is more active in an acid substrate Cobra venom is after all a digestive secretion Another possible explanation may be that cobra venom solutions in distilled water have an alkaline reaction and are, therefore, negatively charged If the venom comes in contact with a paramecium culture of a lower pH (i e, positively charged), the positive and negative ion of the two solutions will attract one another with great affinity Since the surface charge of the protozoa approximately tend to represent the same pH as that of the medium they are in, they will have more cobra venom depositing upon them and will die sooner if they were acid in reaction

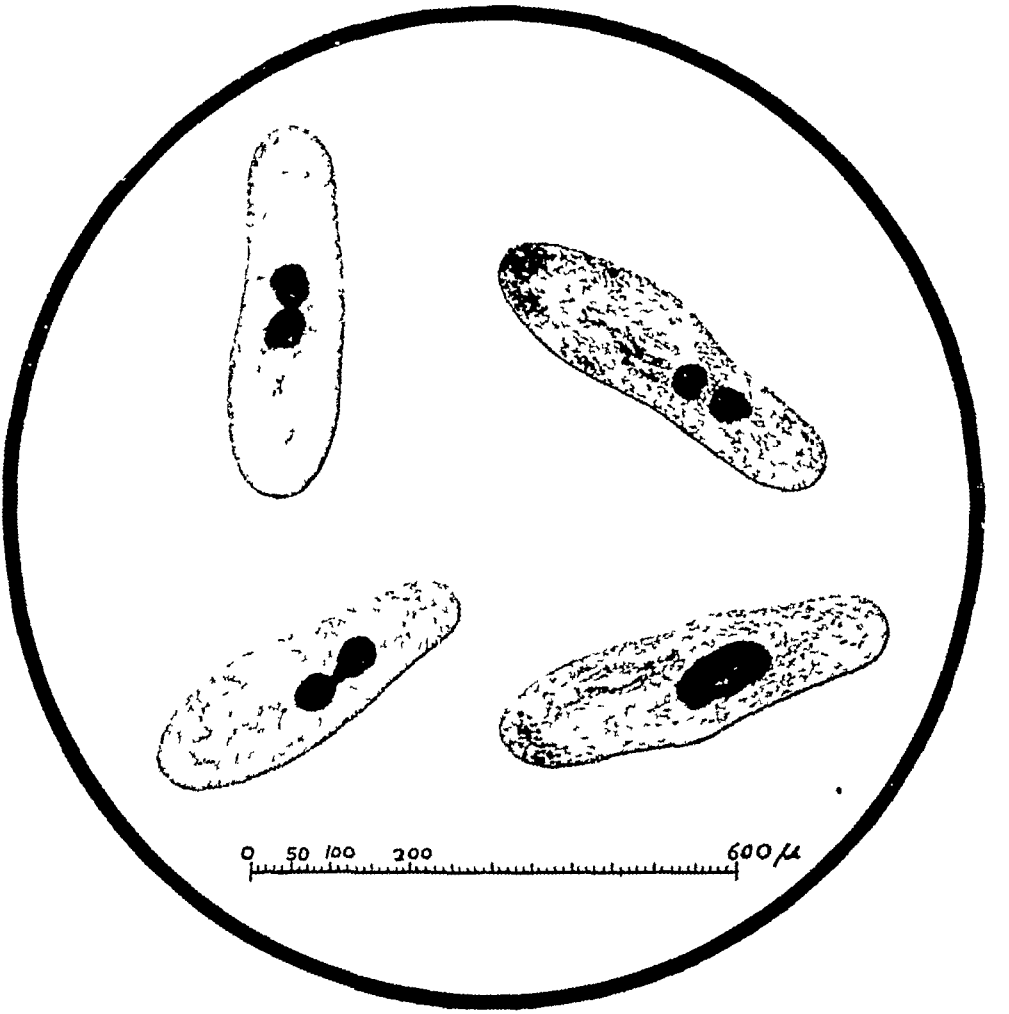
As regards the paralytic action of the venom on the paramecium, McDonald (1922) pointed out that a unicellular organism such as *Balantidium coli* has a neuromotor apparatus consisting of a motorium or a coordinating centre with a system of fibrils which can be traced to ciliary roots Is it possible that a similar neuromotor mechanism exists in the paramecium and that the neurotoxic portion of venom acts on this apparatus which is the precursor of the nervous system of multicellular organisms?

Summary

1 The venom of *N Naia* (Indian cobra) has a toxic action on the protozoal organisms The movements of *P caudatum* are slowed after a momentary stimulation and they then become paralysed and sink to the bottom After a time a blister appears on the surface of the ciliate which bursts and the organism entirely disappeared

2 In dilutions up to 1 in 8,000, the venom has a very toxic action on *P caudatum* and kills them in less than half an hour Weaker dilutions up to 1 in 30,000 slow and later paralyse the movements, the organisms have a tendency to clump together, death takes place after an exposure of 1 to 2 hours Some individuals are much more resistant to the effect of the poison than others.

PLATE LVIII



The structure of the normal *P caudatum* Note the well-defined nuclei and the protoplasmic structures (Iron hæmatoxylin stain) Carl Zeiss × 10 ocular, 1/6 objective

PLATE LIX

The structure of the *P caudatum* exposed to the Cobra Venom solution (1—2,000) Note the swollen, hazy and broken appearance of the protozoa and the karyorrhexis in process (Iron hæmatoxylin stain) Carl Zeiss × 10 ocular, 1/6 objective

3 Other protozoal organisms such as *Bodo caudatus, Trichomonas hominis, E histolytica* and *E limax* are much more resistant

4 *P caudatum* show interesting movements known as 'avoiding reaction' when in contact with cobra venom

5 The hydrogen-ion concentration of the substrate has a very important effect on the resistance of the paramecium against cobra venom The lowering of the pH below 7 decreases the death interval, while it increases with the rise of pH above the neutral limit

We are greatly indebted to Lieut-Colonels H W Acton and R Knowles for their advice and help in this research

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AN EXPERIMENTAL INVESTIGATION INTO THE ACTION OF THE VENOM OF THE INDIAN COBRA— *NAIA NAIA VEL TRIPUDIANS*

BY

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THE subject of snake venom is of special interest to India While the total mortality per annum from rabies in this country, including both treated and untreated cases, amongst a population of over 350 millions is at the most 1–2 cases amongst Europeans, and not more than 500 among Indians, the number of deaths annually from snake-bite according to the mortality returns average between 20,000 to 25,000 or more The number of fatal accidents in other parts of the world amounts to from 5–10 thousands annually

The investigations on snake venom carried out during the last thirty years, so far as India is concerned, have been mainly along the lines of classification of the variety of venomous snakes, preparation of antivenin and its keeping properties, the chemical and biochemical features of the venom and its toxicity Interesting observations have been made in America, Brazil, Japan and Germany on the neurotoxic and hæmolytic principles of the venom Much of the difficulty of the workers lay in the variations in the composition and relative proportion of the active principles of the venom as mentioned by Flexner and Noguchi (1902) That the two principles were distinct was shown by the studies of Weir Mitchell and his collaborators (1886) Since then the hæmolytic principle has been fairly thoroughly investigated at least in many of the colubrine and viperine poisons The neurotoxic principle has

or cornea does not produce any anæsthetic effects, nor are there any signs of local inflammation This is rather interesting as the poison of some of the African snakes has a very irritant action on the tissues, severe inflammation is set up in the conjunctival sacs when the poison gains access to them The venom of the Indian cobra is not absorbed to any appreciable extent from the intact mucous membrane This is probably the reason why the method adopted by snake charmers in this country of sucking the venom away from the wounds by mouth produces no ill effects

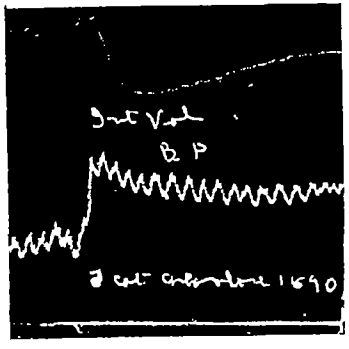
Digestive system—The action of the venom on the functions of the gastro-intestinal digestive enzymes of man was studied The venom by itself *in vitro* was found to have no proteolytic or amylolytic action in dilutions of 1 in 500, 1 in 1,000 and 1 in 2,000 It did not in any way effect the activity of the salivary, gastric and pancreatic secretions of man at different hydrogen-ion concentrations The cobra venom is, however, a digestive enzyme and it undoubtedly helps the activity of other digestive juices in *Naia naia* even though it may not have a digestive action itself It is well known that the reptile dies if all the venom is milked and a bolus of meat is introduced into the stomach The action may be of the nature of a kinase We hope to deal with the digestive properties of venom in a subsequent paper

The venom in doses of 0 1 to 0 8 mg slightly increases the tone of the involuntary muscles of the gastro-intestinal tract but the peristaltic movements are not appreciably increased

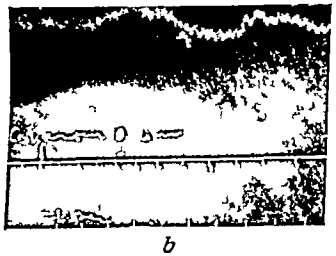
The volume of the intestines is very markedly decreased with small sub-lethal doses (Graph 1, *a*) This is no doubt due to the stimulation of the vaso-motor centre which is discussed in detail later

Action on the circulatory system—The previous work on the action of cobra venom on the circulatory system is indefinite and conflicting According to Manwaring and T B Williams (1923) the action of the venom on the heart is varying and a reliable index of its action is difficult to obtain The most constant reaction was (1) depression of the myocardium after large doses as shown by a decrease in the amplitude of the heart, occasionally preceded by a preliminary period of stimulation, (2) increase of myocardial tone as shown by a rise in the curve occasionally preceded by a decrease of tone These authors further observed that (1) the isolated rabbit's heart showed a distinct resistance during the summer months and a distinct hypersusceptibility during the winter months, (2) hypersensitiveness from recurrent infection and (3) hypersensitiveness from previous toxic injuries

Our experiments were mainly done on cats, dogs and rabbits also were used The animals were anæsthetized with chloralose 0 1 g per kilo body-weight given into the stomach or by intramuscular injections of 1 8 g of urethane Injections of 0 3 to 0 5 mg of the venom to animals weighing roughly 2 kilos (1/10 of the lethal dose) invariably produced respiratory distress The respirations stop for a moment become deep, irregular and spasmodic and this obscured the circulatory effects Urethane cats sometimes showed a fall



a



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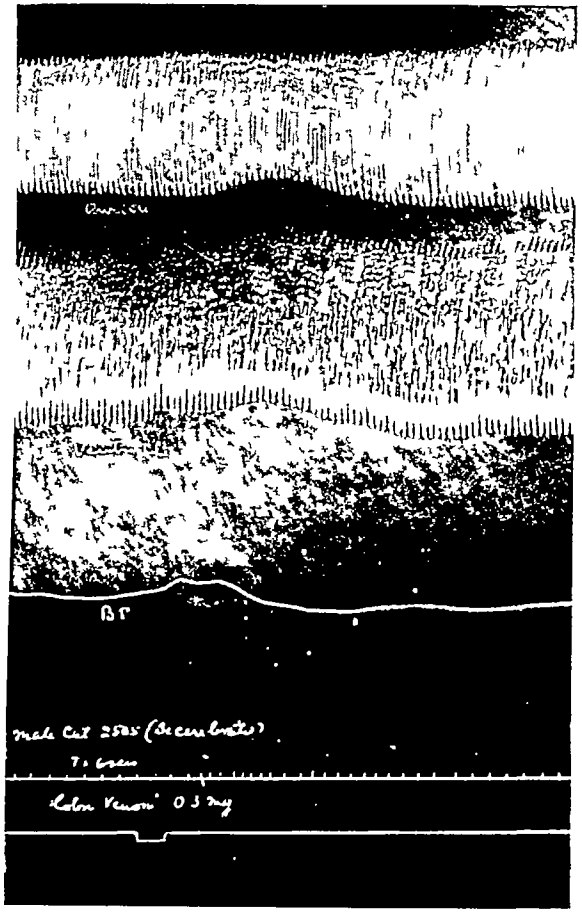

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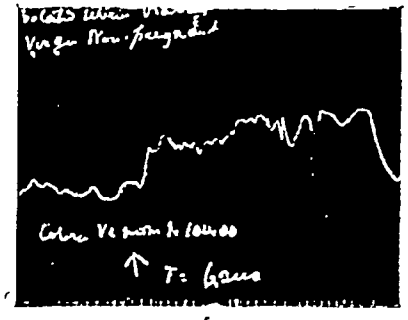
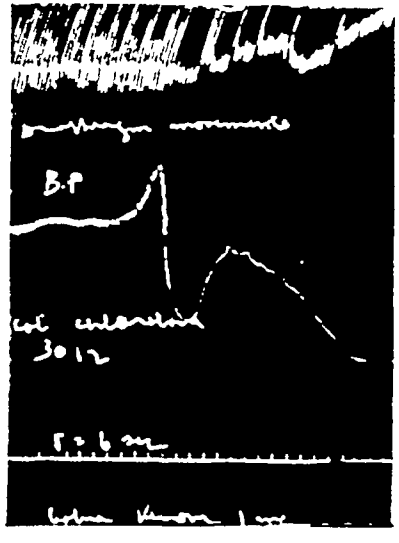


f



g

a Chloralose cat Blood pressure and intestinal volume Note the rise of blood pressure and the corresponding fall in the volume of the intestine

b Urethane cat Sollmann and Pilcher's experiment Upper blood pressure middle record of drops from vein of the isolated limb and lower time Note retardation of flow showing constriction of vessels after intravenous injection of cobra venom

c Isolated kitten's heart Note slight stimulation sometimes produced by venom (1 in 125 000)

d Decerebrated cat Myocardiograph Note slight initial stimulation of the auricles and ventricles There is no rise of blood pressure

e Chloralose cat Chest open and artificial respiration Myocardiograph tracings of auricles and ventricles and blood pressure 2 mg of cobra venom given intravenously Note irregularity and depression of heart and its final stoppage in systole There is tremendous fall of blood pressure

f Isolated rabbit's uterus Note increase of tone by 1 in 10 000 of the venom

g Chloralose cat Chest open and artificial respiration Diaphragmatic movements and blood pressure 1 mg of the venom intravenously produced spasm and increase in tone of the diaphragm and tremendous fall in blood-pressure

and sometimes a rise of blood-pressure, but in either case if the animal was given artificial respiration the action on the blood-pressure tended to be uniform and consistent A dose of 0 3 to 0 7 mg of venom invariably produced a small rise of blood-pressure Up to a certain point the blood-pressure rose in proportion to the dose With the rise in blood-pressure the myocardiograph tracing sometimes showed slight stimulation of the auricle and slight dilatation of the ventricle to begin with, followed by slight acceleration of the heart's action (Graph 1, *d*) This effect is most probably physiologic compensation brought about through the blood vessels and not on account of any direct stimulant action on the heart With larger doses there was often a marked fall in blood-pressure, with obvious cardiac embarrassment There is slowing and weakening of the heart If the dose was not too large the blood-pressure in most cases had a tendency to return to normal after a short time probably due to the venom passing out of the heart and getting diluted in the general circulation (Graph 2, *d*) With toxic doses (1 5 mg or more) the fall in blood-pressure was extreme and permanent and the heart never revived (Graph 1, *e*) The fall of blood-pressure with large doses is mainly due to paralysis of the vaso-motor centre In decerebrated animals (in whom the brain was destroyed but the spinal cord was left intact) kept alive with artificial respiration, the blood-pressure showed little or no changes (Graph 1, *d*) It would appear from this that the effects of the venom are probably central in origin

The heart—The heart in myocardiograph experiments apart from the compensatory effects due to rise in blood-pressure, brought about by vascular changes, did not show any appreciable effects with such doses as 0 3 to 0 8 mg With toxic doses such as 1 to 1 5 mg and more, the heart stopped in a short time, the auricles and the ventricles at first beating slowly and irregularly and finally stopping in a condition of partial systole (Graph 1, *e*) In decerebrated animals 0 5 to 0 8 mg of the venom produced no fall in blood-pressure and the myocardiograph showed little or no change in the auricular or ventricular contractions Toxic doses, however, produced similar results to those described above

The volume of the heart recorded by cardiometer showed slight dilatation of the heart after such doses as 0 3 to 0 8 mg, probably due to the rise in blood-pressure already referred to This effect appears to be compensatory to accommodate the blood pushed into the heart by constriction of the blood vessels

The effect of the venom was also studied on the isolated heart of kittens and rabbits Dilution of 1 in 20,000 and upwards did not produce any marked effect on the kitten's heart With stronger concentrations the heart was depressed and with still higher concentrations (such as 1 in 5,000) the heart stopped This effect in all probability was produced by large quantities of foreign colloidal matter passing into the cardiac capillaries or possibly an enzyme action None of the concentrations of the venom, however, high or low, produced any definite stimulation of the heart either by its action on the

sympathetic or direct action on the myocardium though rarely, especially when the heart is quite fresh, slight augmentation of the beat is sometime observed (Graph 1, c) When the heart is tired or is failing, cobra venom solutions never stimulate it

In the case of the isolated heart of rabbits the venom in dilutions of 1 in 100,000 and more did not show any effect With concentrations of 1 in 50,000 there was a gradual depression of the heart action With higher concentrations such as 1 in 20,000 the heart's action was considerably depressed in most instances and the heart never revived In case of the rabbit's heart and also that of the cat no concentration of the venom showed any definite signs of stimulation of the heart

It will be seen, therefore, that the rise of blood-pressure after injections of the venom is not due to stimulation of the accelerator mechanism or of the myocardium The rise of blood-pressure appears to be associated with the medullary centres as it was absent in decerebrated animals when these centres were destroyed That the centre in the medulla is responsible for the pressor effect produced is supported by the following experiment It should be noted at the outset that the vaso-motor centre is very sensitive and any adverse influence such as excessive haemorrhage or shock due to rough handling or excessive damage to the tissues may interfere with the proper functioning of the centre and vitiate the results The animal, therefore, need very careful handling

A cat under anæsthesia was given artificial respiration The circulation of the right limb was isolated by ligaturing the abdominal aorta about the level of the fourth lumbar and femoral artery and the vein of this limb were divided (Sollman and Pilcher, 1910) This limb was then perfused by putting a canula into the cut peripheral end of the femoral artery, the inflow of the perfusing fluids being measured by the entry of the air bubbles into the mariot's bottle containing oxygenated warm Ringer's fluid and defibrinated blood The circulation in this limb was thus entirely isolated from the rest of the body and the outflow from the limb could be recorded on a drum moving with moderate speed by an electric recorder If now cobra venom is injected into the left femoral vein it will circulate in all other parts of the body except the right limb and any effect produced on the vessels will not be direct but through the centre in the medulla, as the nervous connection of this limb are still intact Injections of cobra venom intravenously in such animals produced a distinct contraction of the vessels as evidenced by retardation of the entry of perfusing fluid (Graph 1, *b*) This effect could be brought about through the agency of the centre in the medulla or ganglia, if any, above the limb since the blood supply to the right leg was cut off

Respiratory system—That death from cobra poisoning is primarily due to failure of the function of respiration has been known for a long time A J Clarke (1927) mentioned that the local effects of cobra venom are not marked but the toxin produces a general central paralysis and death is produced

from paralysis of respiration Cushney (1916) on the other hand maintained that paralysis of the motor nerve endings of voluntary muscle is the main cause According to Heffter (1926) the respiratory centre is paralysed first In warm blooded animals the respiration stops and there is also the curari-form action side by side In some cases perhaps there is paralysis of the endings of the phrenic nerve

Very valuable work has been done by Charles J Martin and George Lamb, and some of the workers in Brazil like Edwin Faust who, while analysing the active principle of snake venom, found that it contained (1) a neurotoxin which had a special affinity for the nerve cells and particularly for the cells of the respiratory centre, (2) a neurotoxin with affinity for the nerve termination of the muscles and particularly for those of the diaphragm These special affinities vary according to different poisons Edwin Faust maintains that the neurotoxin which has an inhibiting action on the nerve termination of the muscles, especially on the phrenic nerve of the diaphragm, is entirely independent of that which acts on the cells of the respiratory centre inasmuch as there are poisons of reptiles such as *Notechis scutatus, Vipera berus, Crotalus adamantus,* etc, which produce inhibitory bulbar action yet do not cause diaphragmatic inhibition Acton and Knowles (1914-15) stated that the use of artificial respiration was confined to cases of colubrine bite and that there is no need for its employment in the case of viper venom where death takes place from cardiac failure and later on from multiple hæmorrhages

From a perusal of the above literature it would appear that the exact site and nature of action of the venom on the respiratory system has not been definitely ascertained We tried to elucidate this by devising a series of experiments

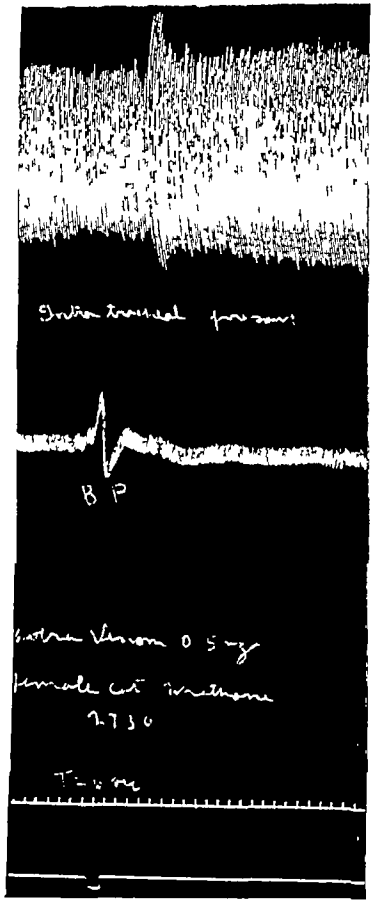
Cats anæsthetized with chloralose or urethane were generally used in these experiments The action of the venom on the lung capacity or lung volume was first determined It was found that in cats under chloralose anæsthesia the action on the respiratory centre was so preponderant that it obscured any effect that might be produced on the bronchioles The animals were, therefore, anæsthetized with urethane, which has neither a marked stimulant nor a depressant action on the respiratory centre In a decerebrated cat the intra-pleural pressure was recorded by introducing a canula into the pleural cavity and recording the movements by a tambour It was noticed that there was often no effect or a very slight constriction of the bronchioles as shown by diminished excursions of the lever A second or third dose of the venom in the same animal did not elicit any response Measurement of intra-tracheal pressure in a cat under urethane (without artificial respiration) by directly connecting the tracheal canula to the tambour, showed that there was acceleration of respiration (Graph 2, *b*) Sometimes there was a short stage of apnœa followed by convulsive spasms (Graph 1, *b*) In view of the diversity of results obtained we tried to determine the part played by the medullary centres In order to do this the following experiment was performed The

abdomen was opened and the diaphragm was carefully separated from its costal attachments An opening was made into the chest in the xiphi-sternal region to get sufficient room to isolate the phrenic nerves on either side The strip containing the phrenic nerve endings on the left side of the animal was secured, making sure that the attachment behind was as tendinous as possible so as to cut off all blood supply This method is not an ideal one as according to Earl Thomas and F E Franks (1928) certain amount of blood circulation must still go on In animals like the dog the strip containing the phrenic nerve endings can be completely separated from the rest of the diaphragm and movements of the diaphragm can be studied by keeping one end fixed and connecting the other end to a lever by a suitable system of pullies In the case of the cat there are difficulties as the phrenic nerve is not long enough to permit isolation of the strip of diaphragm Besides the diaphragm slip is often too small to be fixed at one end and connecting the other end to a recording lever Having ligatured all the blood supply to the diaphragm the movement were recorded This experiment was performed in both the cat and the dog The movements of the diaphragm and the costal muscles are dependent on several factors and in our experiments the possibility of respiratory centre not functioning when the animal is under artificial respiration was not overlooked The centres in the medulla must be efficiently supplied with proper quality of blood to function properly Over-ventilation of the lung, by increasing oxygen tension in the blood tended to cause apnœa and under-oxygenation by increasing the CO_2 tension, though to start with stimulated the respiratory centre, tended to stop the heart by acting adversely on the heart muscle A careful manipulation of artificial respiration to maintain satisfactory diaphragmatic and costal movements is therefore essential After all these factors are carefully attended to, both the diaphragmatic and costal movements can be recorded on the drum Smaller doses such as 0 4 mg in such a preparation produce a marked acceleration of the diaphragmatic movements and a definite increase of tone (Graph 2, *c*) Larger doses of the venom produced a definite cessation of respiration with a spasmodic condition of the diaphragm (Graph 3, *a*) In some cases the respiration became spasmodic and jerky The costal movements were also recorded along with the diaphragmatic movements by attaching one side of the chest to a lever through a system of pullies It was shown that injections of the venom affected both the costal as well as the diaphragmatic movements (Graph 2, *d*) The venom must, therefore, act on one or more of the following (1) the centre in the medulla which controls the diaphragmatic and costal movements, (2) on the nerve endings in the muscle concerned, or (3) on the muscle fibres

To see if the venom was acting on the nerve endings of the phrenic, we put the centre out of action either by sectioning the phrenics or temporarily paralysing the nerves with CO_2 snow The movement stopped at once

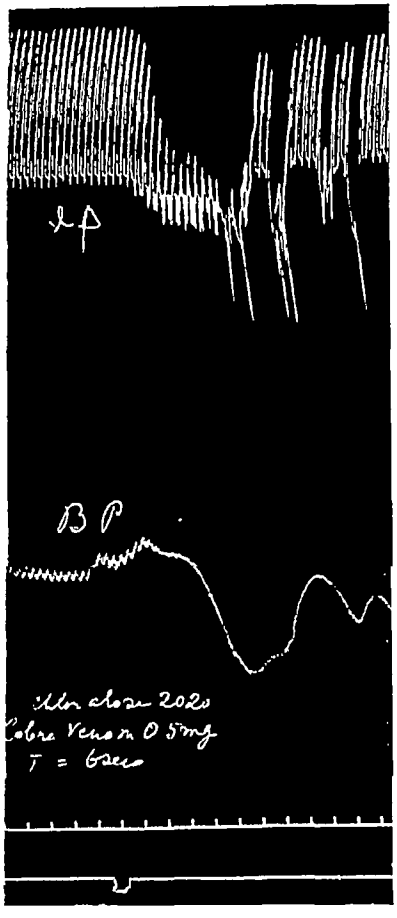
In another experiment we retained the blood supply to the diaphragm by not separating the right half of the diaphragm from its costal attachment,

Graph 2



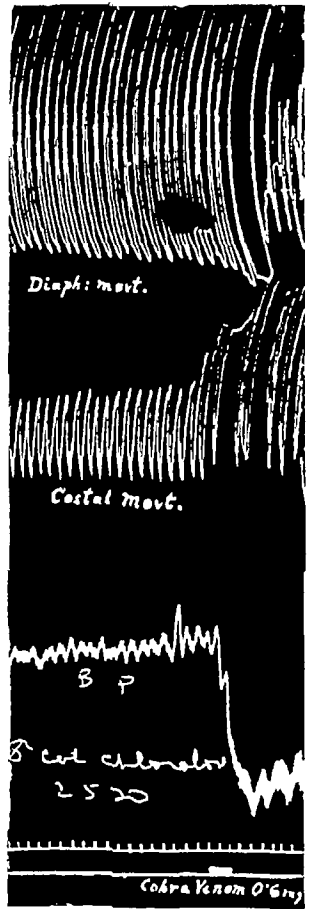
a

a Urethane cat Intratracheal pressure and blood pressure 0 5 mg of the venom intravenously produced stimulation of the respiratory movements



b

b Chloratone cat Intratracheal pressure and blood pressure Note apnœa and spasmodic respiratory movements after intravenous injection of 0 5 mg of the venom



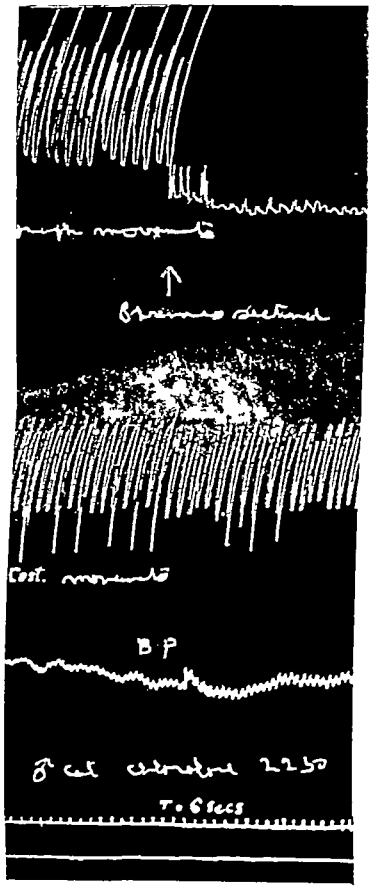
c

c Chloralose cat Chest opened and artificial respiration Record of movements of strip of diaphragm and blood pressure 0 4 mg of the venom given intravenously produced marked acceleration of movements and increase of tone

d

d Chloralose cat Chest open and artificial respiration Record of strip of diaphragm costal movements and blood pressure 0 6 mg of the venom produced fall of blood pressure and irregularity of movements

Graph 3

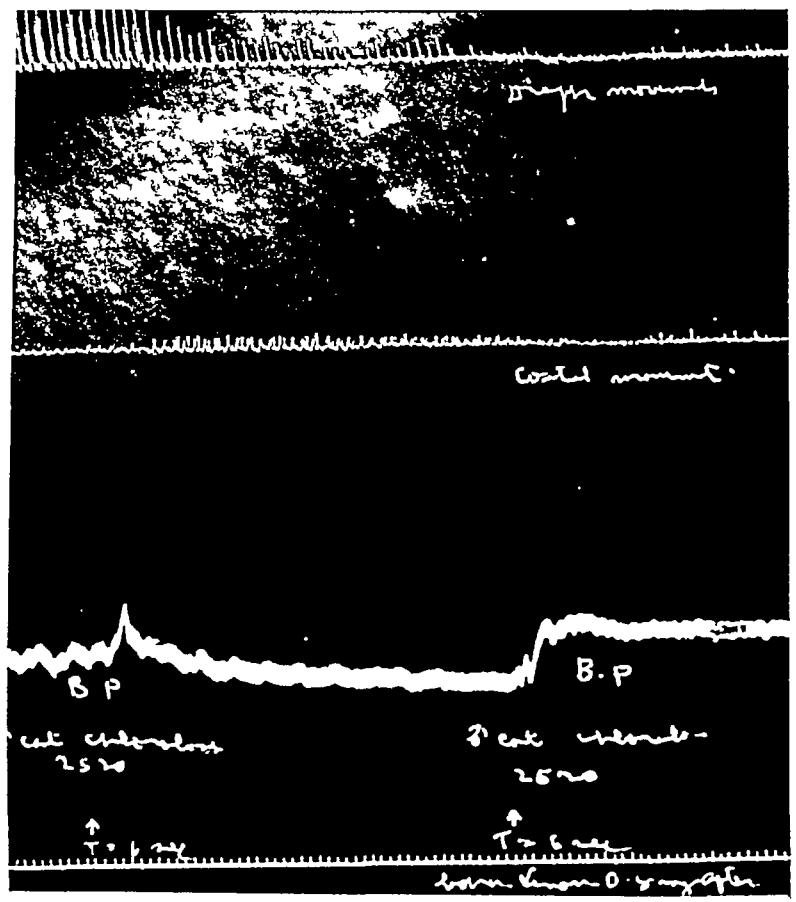


a

a Chloralose cat Chest open and artificial respiration From above downwards diaphragmatic movements, costal movements and blood pressure At phrenics were cut Note stoppage of diaphragmatic movements while costal movements go on

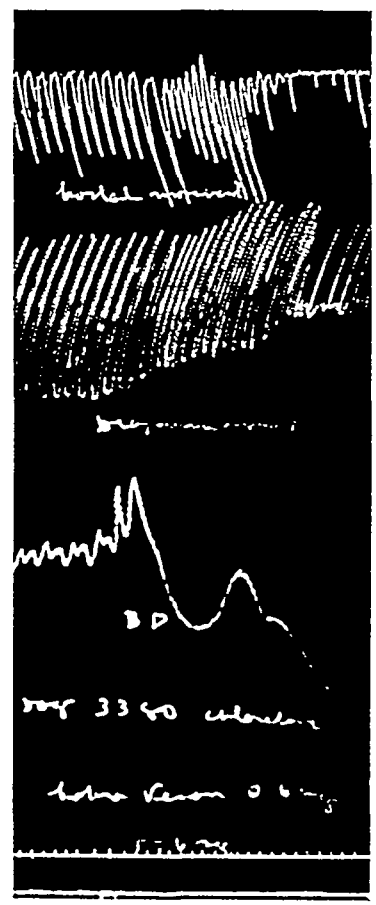
b

b 5 minutes after section of phrenics 0 7 mg of venom given intravenously Note effect on costal movements



c

c Chloralose cat Chest open and artificial respiration From above downwards record of diaphragmatic and costal movements and blood pressure Effect of venom after paralysis of end plates with curare



d

d Effect of venom on costal and diaphragmatic movements in dog after 0 6 mg of the venom

The strip from the left half was just made up for convenient fixing of the recording lever, otherwise the blood supply was as far as possible permitted to continue Section of the phrenic or freezing completely paralysed the respiratory movements of the diaphragm, the muscle losing its tone and becoming relaxed while the costal movements continued (Graph 3, *a*) Injection of cobra venom at this stage produced no effect on the movements of the diaphragm but had the usual effect on the costal movements (Graph 3, *b*) This shows that the effect produced was not due to any action on the part of the venom on the endings of the phrenic nerve or on the muscle of the diaphragm

We next paralysed the motor nerve endings of the respiratory apparatus by injecting 4 to 5 mg of curare It was noticed that in the cat the costal movements were paralysed earlier than the diaphragmatic movements by this alkaloid This may be due to the fact that the nerve endings in the diaphragm are more resistant to this drug than those in the costal muscles or perhaps diminished blood supply to the diaphragm was the cause for delayed effect If at this stage a dose of cobra venom is given, the diaphragmatic movements are distinctly stimulated whereas the paralysed costal movements are not affected If a second dose of 4 mg of curare is now given both the costal and the diaphragmatic movements are paralysed, the administration of the venom now produces no change in the movements of either (Graph 3, *c*) This experiment further corroborates the view that the action of the venom is central and that it has no action on the nerve endings of either the diaphragm or the muscles responsible for costal movements The isolated strips of the muscular portion of the diaphragm with the phrenic nerve endings intact were perfused in Dale's uterine bath No rhythmic movements in the diaphragm were noticed as occur in some of the other muscular tissues Addition of cobra venom in dilution of 1 in 1,000 slightly increased the tone of the muscle as shown by the rise of the lever Lower concentrations produced no effect whatever

The experiments on the dog and rabbit showed somewhat similar effects (Graph 3, *d*) In case of the rabbit doses of 0 2 to 0 4 mg per kilo affected both the costal and diaphragmatic movements producing deeper respiratory movements at longer intervals Further a single dose invariably permanently affected the respiratory mechanism in a rabbit

The action of the venom on the higher centres and on the nerve endings associated with respiratory movement has been debated In the cat at any rate the action on the phrenic nerve endings appears to be negligible as shown by the following experiment An animal whose diaphragmatic movements were being recorded with the phrenic nerve isolated was stimulated with a weak current from a Du Bois Raymond secondary coil to elicit a response from the diaphragm The minimum strength required to produce a contraction being determined the animal was given a fairly large dose of cobra venom, namely 1 mg per kilo, which showed the usual effect on the respiration, the

diaphragm and the heart The phrenic nerve was then stimulated from an inductorium with the minimum strength which gave a response before the venom was given This stimulus still produced a response showing the end plates in the diaphragm were not adversely affected by the venom When curare is given, which paralyses the end plates, absolutely no response is produced by application of stimulus to the phrenics In the rabbit the venom has been said to act mainly on the phrenic nerve endings but in our experiment the dose necessary to cause a block in the phrenic nerve also stopped the costal movements Stimulation of the phrenic nerve with an interrupted current still gave a response showing that it is the centre in the medulla which is affected before the paralysis of the ending of the phrenic even in a rabbit

Voluntary muscle—The action of the venom on the muscle and nerves was studied in a muscle nerve preparation of a frog by comparing the strengths of current which produced a contraction before and after the application of the venom It was shown that the tone of the muscle was slightly increased by the venom in dilution of 1 in 1,000 In mammals it was observed that direct application of the venom increased the tone but this was not the case when it was administered intravenously Probably the concentration necessary for increasing the tone of the muscle is very high and cannot be attained by intravenous administration It was shown that the venom even in high concentration had no paralytic effect either on the nerve endings in the muscle or muscle itself

The movement of isolated uterus recorded in Dales' uterine bath showed increase of tone in concentrations of 1 in 5,000 to 1 in 10,000 but the automatic movements were not appreciably affected (Graph 1, *f*)

Central nervous system

We have fully discussed the action of cobra venom on the vaso-motor and respiratory centres The action of the venom on the central nervous system was studied on animals who had received lethal and sub-lethal doses Animals such as the cat and the rabbit become apathetic after a preliminary stage of excitement looked drowsy, the respirations became hurried and later there was slight paralysis of the hind limb–the animals attempted to move dragging the hind legs They were lethargic and showed no desire for food Some of the previous workers have described general curariform effect of the cobra venom but this was not borne out by our experiments No paralysis of the end plates could be demonstrated by testing the nerves by means of interrupted current either Biochemical work of Edwin Faust, Charles Martin and George Lamb has shown that the separated neurotoxic principle affected the respiratory centres in the medulla and not the cerebral cortical cells Clinical observations in man after cobra-bite show that the effects are similar to those observed in animals The patient has drooping of the eyelids (ptosis) and complains of weakness in the legs, is lethargic and shows no desire to move There is inability to speak and sometimes the pharyngeal muscles are paralysed

and the patient cannot drink water as in hydrophobia Some of the physiologists have drawn attention to the possibility of a substance that stimulates the medullary centre in a mammal depressing the cortical centre, and vice versa It is probably on this basis that recently the venom of some snakes especially those belonging to the viper family has been used as a cerebral sedative and in the treatment of epilepsy Our observations on animals have so far not shown any justification for this belief

Discussion

That the action of cobra venom varies with the relative proportion of various constituents of the poison is established beyond question The neurotoxic principle in the Indian cobra produces respiratory effects almost entirely through the respiratory centre and not through the nerve endings This has been clearly brought out by our experiments The absence in a pithed animal of both the circulatory and respiratory effects shows that the centres in the brain are entirely responsible The heart muscle is not affected by ordinary doses Neither the perfusion of the heart with various concentrations of the venom nor the myocardiographic experiment showed any stimulation An appreciable rise in the blood-pressure is generally observed provided the respiratory effects does not obscure it That this rise in the blood-pressure is entirely due to stimulation of the vaso-motor centre has been proved by the use of Sollmann and Pilcher's technique The venom here is not permitted to reach the limb perfused so it cannot act locally That the effect on the respiration is also mainly due to its action on the centre in the medulla is shown by (1) its absence in pithed animals, (2) by its absence in the diaphragm when the phrenics are cut or frozen while it still persists in the costal muscles, and (3) by its absence in both diaphragmatic and costal movements after administration of paralytic doses of curare The action on the centre, especially with small doses, is at first stimulation as shown by increase in the tone of the diaphragm and other muscles concerned in the respiratory movements The respiratory efforts become deep and spasmodic Paralysis of the centre comes on later With large toxic doses the stimulant effect is often not noticeable As disturbances in the CO_2 tension may also produce stimulation of the centre, in our experiments the artificial respiration was maintained at an even tension of oxygen and CO_2 so that this factor did not come into play

As regards the paralysing action of the venom on the endplates in the diaphragm emphasised by many workers, it has been shown in the case of Indian cobra venom at any rate, that stimulation of the phrenic nerves with an interrupted current of known strength produces an equal response in animals who have been given multiple lethal doses of the venom and in those who have not had it If these end-plates are paralysed by curare no such response is obtained From all these data one is justified in concluding that the venom of *Naia naia vel tripudians* stimulates the medullary centres in small doses, i e, the respiratory and the vaso-motor, the effect on the respiratory centre

being somewhat more powerful than on the vaso-motor centre Large doses produce a paralysis of both the centres Since the vagal centre is in the same region of the medulla there is a possibility of the venom stimulating the vagus centre as well, though the manifestation of the stimulation of the vagal centre in the heart, the bronchioles, and in the intestinal movements is not evident with ordinary doses

Summary and conclusions

1 The M L D of the venom from Indian cobra *Naia naia vel tripudians* varies with the species of animals, cats and rats are less susceptible, dogs, rabbits and man are more easily affected

2 When given intravenously the venom produces an immediate effect, the animal dying within a few minutes of respiratory failure provided a large enough dose is given The absorption is slower when the venom is given by the subcutaneous and intramuscular routes, death taking place in 4 to 24 hours The venom is not absorbed at all from the gastro-intestinal tract or other mucous membranes

3 The venom has no effect on the activity of salivary, gastric and pancreatic secretions of man *in vitro* It slightly increases the tone of the musculature of the gastro-intestinal tract in cats and rabbits

4 Injections of sub-lethal doses of the venom produce a small but persistent rise of blood-pressure in experimental animals This rise is not due to any stimulant action on the accelerator mechanism of the heart or on the myocardium None of the concentrations of the venom, however high or low, produce definite stimulation of the heart especially when it is failing Very large doses appears to act directly on the heart producing a marked depression and stoppage

5 The rise of blood-pressure appears to be associated with the stimulation of the vaso-motor centre in the medulla as it is absent in decerebrated animals The fall of blood-pressure produced by large doses has been shown to be due to paralysis of the vaso-motor centre

6 The main action of the venom in lethal and sub-lethal doses on the animals is on the respiratory centre, the effect being one of initial stimulation and final paralysis

7 The venom appears to have no effect on the motor end plates in the diaphragm or other respiratory muscles

8 Observations on animals show that the venom produces initial stimulation of the higher parts of the brain followed by paralysis There appears to be no justification for its use as a cardiac stimulant or in the treatment of epilepsy, chorea, etc

We are very grateful to Lieut-Colonel H W Acton, I M S, for his valuable advice and help in the course of this investigation

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THE EARLY STAGES OF SOME INDIAN MOSQUITOES *MEGARHINUS*

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THE larvæ of *Megarhinus* are very uniform in structure throughout the genus, and, up to the present, no attempt appears to have been made to draw up a synoptic table for the identification of those found in any particular region In the published descriptions of the early stages little or no reference appears to have been made to differences in pilotaxy, it being generally assumed that such did not exist A study of the 4th stage larvæ and of the pupæ of the six Indian species has revealed differences which are constant in the material available and keys are given below for the identification of these stages It seems probable that the characters used in these keys will be of value in the differentiation of larvæ and pupæ of species occurring in other countries

The important structural characters of both the larvæ and pupæ are illustrated on the accompanying plates

The larvæ are of some importance in India as, by their predaceous habits, they serve to keep down the numbers of blood-sucking forms such as *Ædes* (*Stegomyia*), *Ædes* (*Finlaya*), *Anopheles*, and others, with the larvæ of which they are most frequently found in association in tree-holes and bamboo stumps

Larvæ of the 4th instar may be distinguished from those of other genera by the following characters very large larvæ, usually of a deep red or reddish-brown colour, those of the larger species measuring up to 16 mm in length Head sub-quadrangular, hairs on dorsal surface all placed very far

forward, frontoclypeus divided by a suture into an anterior part and a much larger posterior part, the anterior carries four hairs on each side, three fine and simple and one very minute and spine-like (the outermost), the posterior part also carries four hairs on each side, three fine and simple and one very small and branched (the innermost) Antennæ short, shaft smooth, hair tuft represented by two fine hairs situated some little distance below the tip, between these and the tip there is a small branched sub-apical hair Mouth parts adapted for predaceous purposes, hairs of mouth brushes modified into strong lamellæ with hooked tips Thorax with some heavily chitinized plates carrying thick spinulose bristles The arrangement of the thoracic hairs is shown in diagrammatic form on Plate LX Pro-thorax with two large plates on each side, a dorso-lateral and ventro-lateral, the former carries hairs 6, 7, and 8, the first and last being bristles, the latter carries hair 13, always small and branched, and the four pleural hairs (9 to 12) on a raised tubercle, one of these being a bristle On the meso-thorax there are in some species three plates, in others four, on each side, in the former the dorso-lateral plate bears hairs 3 to 7, 6 being a bristle, in the latter the plate is divided, hairs 3 and 4 being on a separate small plate, hair 8 is lateral, bristle-like, and arises from a tubercular plate, the ventro-lateral plate carries the pleural hairs 9 to 12 and hair 13, as on the pro-thorax On the meta-thorax there appear to be thirteen hairs as against fourteen on the pro- and meso-thorax, by comparing the hairs on the three parts of the thorax it would seem that the missing hair is No 7, hairs 6, 8, and 14 are bristles arising from separate plates, the last being ventral, the pleural hairs and hair 13 are on a ventro-lateral plate as on the other two segments In addition to the large plates mentioned there are some much smaller plates from which the finer hairs arise On abdominal segments 1 to 7 there are three plates on each side a dorso-lateral, lateral, and ventro-lateral, which carry either spinulose bristles or long plumose hairs, or both, these are very similar in all the species except the dorso-lateral plate on the 7th segment, on which there may be two bristles and three hairs, or one bristle and four hairs Eighth segment with a large lateral plate on each side in place of a comb, with two strong bristles and some small hairs, the length of the longer bristle compared with the length of the siphon tube is of diagnostic value in some cases Siphon tube short and wide, a single pair of strong branched hair tufts arising near the base, pecten absent Anal segment enclosed in a strongly chitinized ring with numerous spines along the posterior margin, both long and short irregularly alternating, lateral hair bristle-like, spinulose, both pairs of sub-dorsal hairs divided into a number of long branches, anal papillæ quite short, anal fan well developed, the hairs split into fine branches The relative length of the siphon tube* and of the chitinized part of the anal segment† is of importance in certain species

* Measured along the anterior margin in side view not including the valves
† Measured along the dorsal border in side view

side of the thorax are connected by a very narrow strip of chitin, on the opposite side of the larva, in each case, the plate is divided into two Skins of 10 isolated larvæ and four complete larvæ have been examined

Pupa (Plate LXII, figs 17, 20 and 29) of the five sub-median and sub-lateral hairs two are moderately long on tergite 2, and three on tergite 3, two are long and black on tergites 4 and 5, and one on tergite 6, the larger lateral hair on segments 2 to 4 is fine and short, that on 5 and 6 longer, that on 7 quite small The posterior border of the paddle is occasionally only slightly emarginate and the pupa then resembles that of *M gravelyi* closely, the hairs on tergites 1 to 7 having very much the same arrangement and development

M gravelyi Edw *Larva*, 4th stage (Plate LXI, figs 11, 12 and 13) this is fairly easily separated from the larvæ of *M albipes* and *M minimus* (with which it agrees in having the meso-thoracic dorso-lateral plate divided into two) by having the siphon distinctly longer than the chitinized part of the anal segment Length of siphon 1 5 to 2 0 mm , siphonal tuft of four branches in the skins of two isolated larvæ examined

Pupa (Plate LXII, fig 28) differs from all the other species in the shape of the paddle which is not emarginate on the posterior border (in this respect resembling the pupa of *M magnificus* from Malaya) In the character and development of the hairs on the abdominal tergites it agrees closely with the pupa of *M albipes*

M minimus Theo *Larva*, 4th stage (Plate LXI, figs 8, 9 and 10) the comparatively small size and the characters given in the key serve to distinguish this larva from those of the other five species Siphon 0 7 mm long, siphonal tuft, in one specimen, of three branches on each side, in another specimen the tuft is represented by a single strong hair on one side, and by a two-branched hair on the other Skins of two isolated larvæ examined

Pupa (Plate LXII, figs 21 and 27) size and shape of paddle fairly distinctive Of the sub-median and sub-lateral hairs one is long on tergites 2 to 6, and a second of moderate length on tergites 2 and 3, larger lateral hair on segments 5 and 6 very long

M kempi Edw *Larva*, 4th stage (Plate LXI, figs 4 and 5) length of siphon 1 0 to 1 2 mm , siphonal tuft of 4 to 6 branches, more usually 5 Bristle representing hair 8 on pro-thoracic dorso-lateral plate single (in the skins of five isolated larvæ examined), in some other species this bristle is invariably bifid

Pupa (Plate LXII, figs 19, 22 and 24) paddle of very distinctive shape and without an irregular black line across the base (in these characters closely resembling the pupa of *M leicesteri* Theo from Malaya) Of the five sub-median and sub-lateral hairs three are rather long and black on tergites 2 and 3, the innermost of these three on 2 branched, two are long and black on tergite 4, and one on tergites 5 and 6

M splendens Wied *Larva*, 4th stage (Plate LXI, fig 7) this is comparatively easy to identify by the characters given in the key When fully

grown it is from 15 to 16 mm long and of a crimson colour or deep reddish-brown Length of siphon 1 1 to 1 5 mm, more usually about 1 2, siphonal tuft with from 4 to 8 branches, more usually 5 The ventro-lateral plate on the 1st abdominal segment is sometimes divided into two The more heavily chitinized parts of the head and body are of a deep rich-brown Skins of 15 isolated larvæ, and two complete larvæ have been examined

Pupa (Plate LXII, figs 18 and 26) of the five sub-median and sub-lateral hairs one is long and black on tergites 2, 6, and 7, and two on tergites 3, 4, and 5, the larger lateral hair on segments 2 to 7 is long

M. edwardsi Barraud *Larva* 4th stage (Plate LXI, figs 1, 3 and 6) resembles that of *M splendens* in general appearance and size but is distinct on the characters given in the key Siphon from 1 0 to 1 2 mm long, siphonal tuft usually of 5 branches Hair 7 on meso-thoracic dorso-lateral plate usually simple, occasionally split into two towards the tip, but not branched as in *M kempi* Chitinizations of head and body deep-brown Skins of four isolated larvæ have been examined

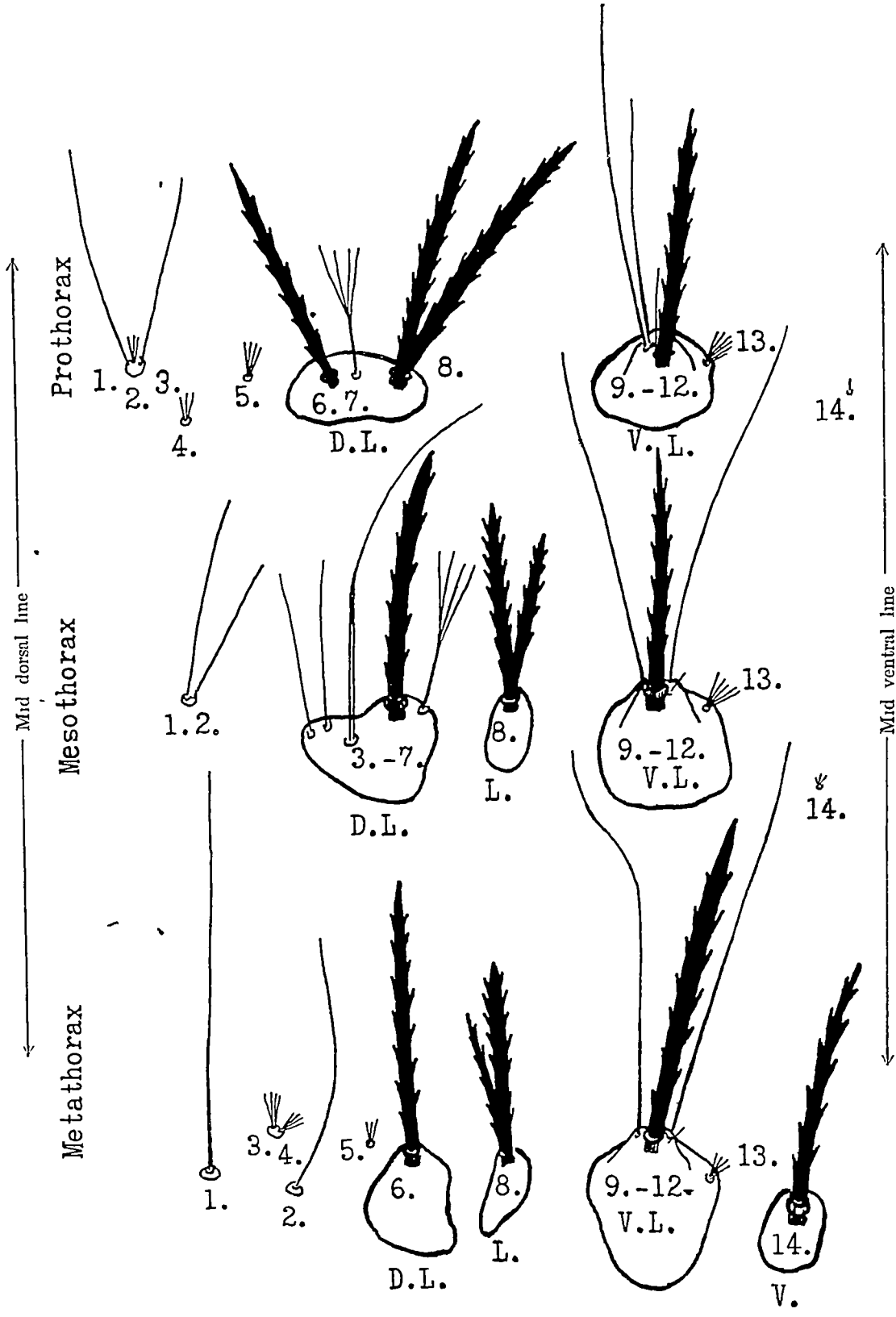
Pupa (Plate LXII, figs 23 and 25) very similar to that of *M splendens* differing only in the length of the larger lateral hair on the 7th segment (*compare* Plate LXII, figs 18 and 23)

In a previous paper (*Ind Jour Med Res*, XVII, 1929, pp 271–280) the adults have been described and the distribution and breeding places given With regard to *M quasiferor* Leic, which was included as an Indian species in Edwards' Synopsis (*Ind Jour Med Res*, X, 1922, p 459), this record was based on a specimen from Sikkim which, on re-examination, proved to be *M splendens*

Explanation of Plate LX

Diagram showing the character and approximate position of the hairs on the right side of the thorax of *Megarhinus* larva (*M splendens* Wied) from the mid dorsal line on the left, to the mid ventral line on the right The numbers from 1 to 14 indicate the individual hairs on each segment of the thorax, 9 to 12 being the pleural hairs in each case On the meta-thorax hair 7 is apparently absent

Lettering —D L Dorso-lateral plates
L Lateral plates
V L Ventro-lateral plates
V Ventral plate


Prothorax
Mesothorax
Metathorax
Mid dorsal line
Mid ventral line
1.
2.
3.
4.
5.
6.7.
8.
D.L.
9.-12.
13.
V.L.
14.
1.2.
3.-7.
8.
L.
9.-12.
V.L.
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14.
V.

Explanation of Plate LXI

Camera lucida drawings illustrating points of structure in the 4th stage larvæ of Indian species of *Megarhinus*

Fig 1 *Megarhinus edwardsi* Barr Meso-thoracic dorso-lateral plate, the hairs shown are, from left to right 3 to 7 the apical part of 5 is omitted

„ 2 *M albipes* Edw Ditto Showing the division of the plate into two parts, hairs 3 and 4 being on a separate small plate

„ 3 *M edwardsi* Barr Pro-thoracic dorso-lateral plate the hairs shown are, from left to right 6 to 8

„ 4 *M kempi* Edw Ditto *Compare* this with Fig 3

„ 5 *M kempi* Edw Meso-thoracic dorso-lateral plate carrying hairs 3 to 7, hairs 3 and 4 being on a projection *Compare* this with Figs 1 and 2

„ 6 *M edwardsi* Barr Dorso-lateral plate of 7th abdominal segment carrying one bristle and four hairs

„ 7 *M splendens* Wied Ditto Carrying two bristles and three hairs

„ 8 *M minimus* Theo Longer bristle from lateral plate of 8th segment *Compare* the length of this with the length of the siphon

„ 9 *M minimus* Theo Siphon tube

„ 10 *M minimus* Theo Chitinized part of anal segment, anal fan omitted, bases of sub-dorsal and lateral hairs indicated

„ 11 *M gravely* Edw Longer bristle from lateral plate of 8th segment *Compare* the length of this with the length of the siphon

„ 12 *M gravely* Edw Siphon tube

„ 13 *M gravely* Edw Chitinized part of anal segment, anal fan omitted, bases of sub-dorsal and lateral hairs indicated

„ 14 *M albipes* Edw Longer bristle from lateral plate of 8th segment *Compare* the length of this with the length of the siphon

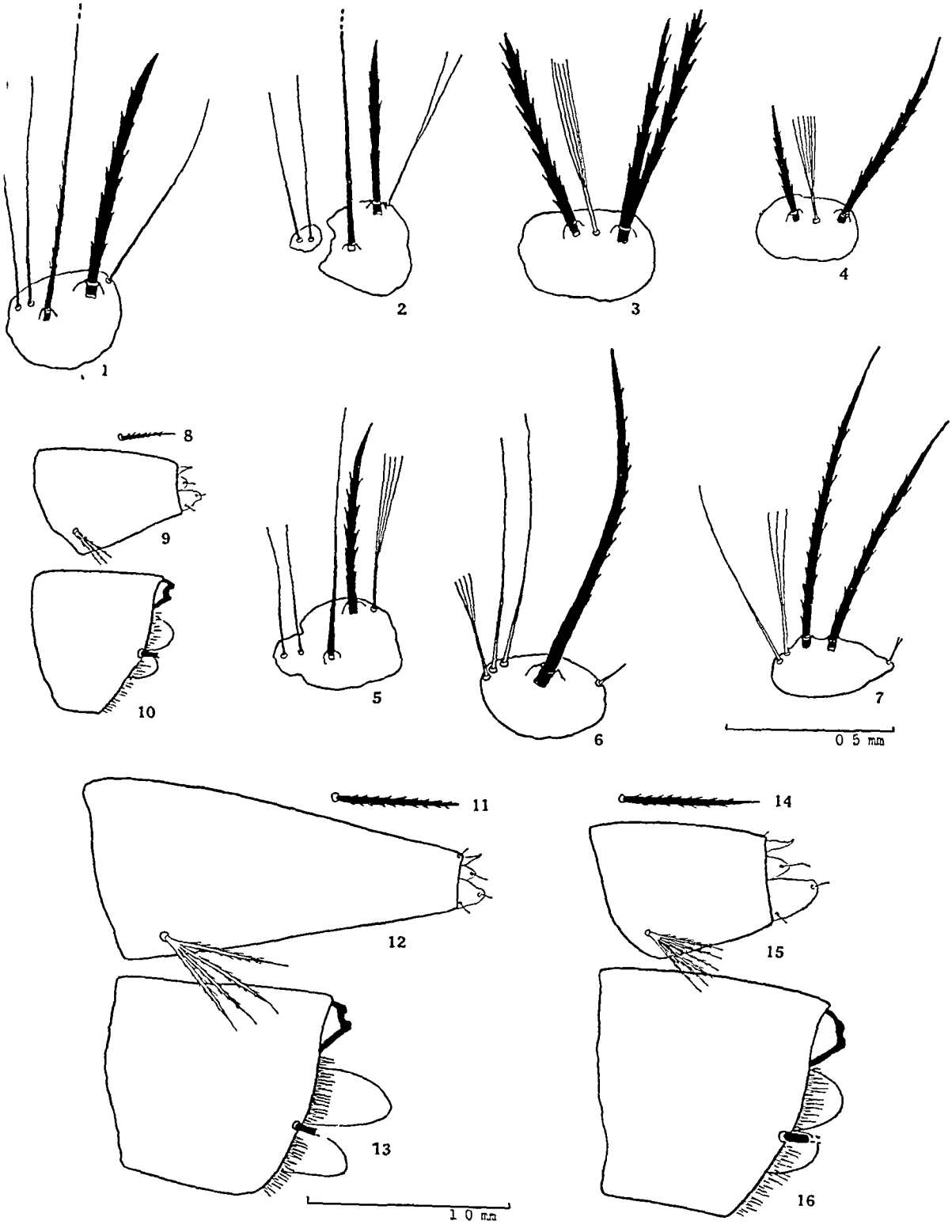
„ 15 *M albipes* Edw Siphon tube

„ 16 *M albipes* Edw Chitinized part of anal segment, anal fan omitted, bases of sub-dorsal and lateral hairs indicated

Figs 1 to 7 drawn to the scale shown under Fig 7

„ 8 to 16 drawn to the scale shown under Fig 13

Plate LXI



Explanation of Plate LXII

Camera lucida drawings illustrating points of structure in the pupæ of Indian species of *Megarhinus*

Fig 17 *M albipes* Edw Posterior border of 7th tergite showing five sub-median and sub-lateral or inner hairs and two lateral

„ 18 *M splendens* Wied Ditto

„ 19 *M kempi* Edw Posterior border of 5th tergite showing hairs

„ 20 *M albipes* Edw Ditto

„ 21 *M minimus* Theo Posterior border of 4th tergite, showing hairs

„ 22 *M kempi* Edw Ditto

„ 23 *M eduardsi* Barr Posterior border of 7th tergite, showing hairs

In Figs 18 to 23 only about one-fifth of the length of the longer hairs is shown in each case

Fig 24 *M kempi* Edw Paddle

„ 25 *M eduardsi* Barr Ditto

„ 26 *M splendens* Weid Ditto

„ 27 *M minimus* Theo Ditto

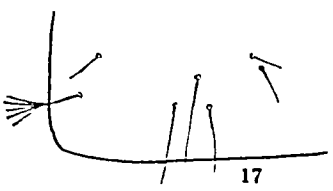
„ 28 *M gravelyi* Edw Ditto

„ 29 *M albipes* Edw Ditto

Figs 17 to 23 drawn to the scale shown under Fig 23

„ 24 to 29 drawn to the scale shown under Fig 29

Plate LXII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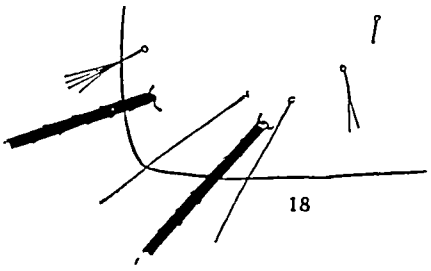
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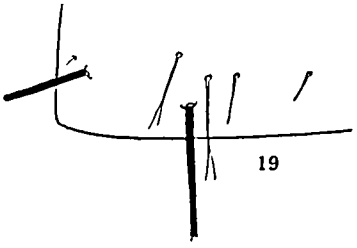
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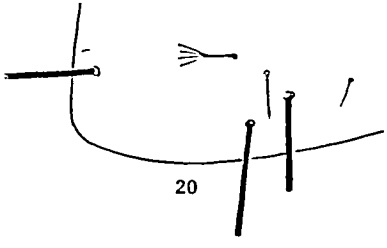
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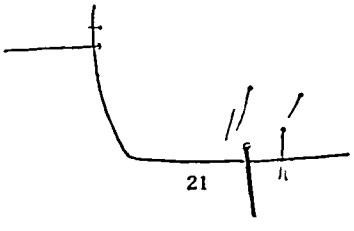
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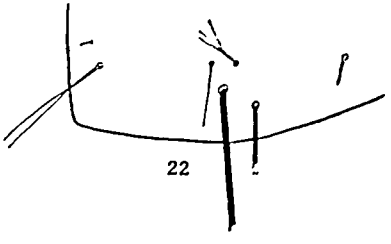
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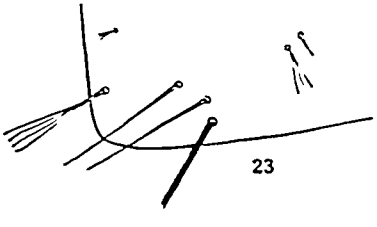
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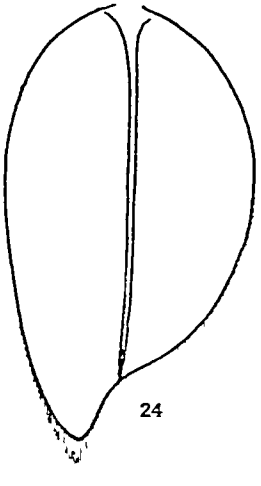
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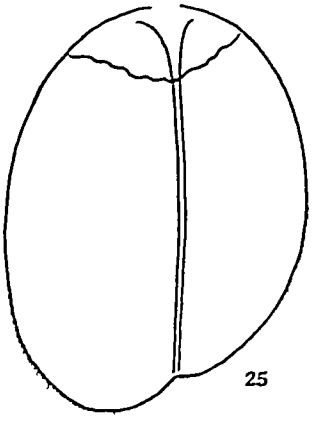
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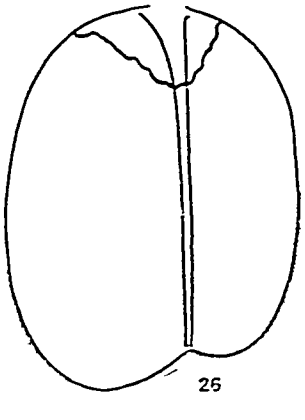
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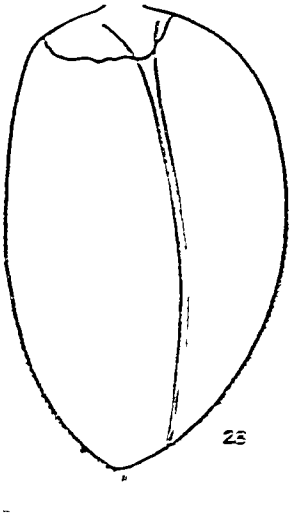
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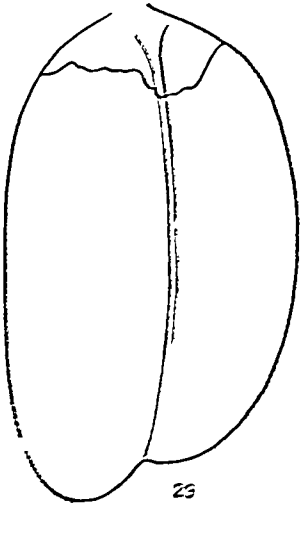
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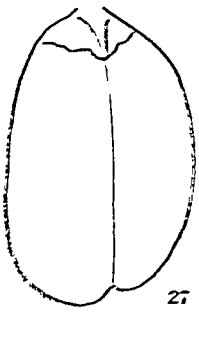
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NOTES ON SOME ANOPHELINE MOSQUITOES COLLECTED IN SIERRA LEONE INCLUDING DIFFERENTIATION OF *ANOPHELES DTHALI* PATTON (MEDITERRANEAN) AS A DISTINCT SPECIES FROM *ANOPHELES RHODESIENSIS* THEO (ETHIOPIAN)

BY

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IN September 1928 one of us (S R C) paid a short visit to Sierra Leone and brought back a collection of Anopheline mosquitoes made in the neighbourhood of Freetown and at Mabang in the Protectorate The collection consisted of adults, with in many cases larval and pupal skins, of *A gambiæ* Giles, *A rhodesiensis* Theo, *A funestus* Giles, *A marshalli* var *freetownensis* Evans, *A theileri* Edw and *A smithii* Theo The results of examination of this material appear of sufficient interest to merit publication of the present note

The collector wishes to express his great indebtedness to Dr R M Gordan, then acting Director of the Sir Alfred Jones Research Laboratory, Freetown, and Dr G Macdonald of the above Laboratory for their extremely kind hospitality and very valuable assistance in many ways The fact that he was able to obtain specimens and larval skins of the very interesting species *A theileri* was entirely due to these officers' local knowledge of the breeding-places of this species in the Protectorate

A (Myzomyia) rhodesiensis THEO

Numerous specimens of this species were bred out from different breeding-places about Freetown Next to *A gambiæ* it seemed at this time to be the commonest Anopheline in the area

Examination of the material has shown that the species called in Sierra Leone *A rhodesiensis* (Blacklock and Evans, 1926, Evans, 1927) differs from the species of which a description is given by Christophers and Khazan Chand (1915) as *A rhodesiensis* from Arabia, etc., and which has been considered this species by a number of authors in Egypt, India and elsewhere, in several important respects, notably in the character of the head-scales and in the presence of scales on the front of the mesonotum, which is entirely free from scales in the eastern form. At our request Mr. Edwards at the British Museum has very kindly examined the type of *A rhodesiensis* Theo. from Rhodesia and he informs us that this resembles the Sierra Leone form in the above respects.

The Sierra Leone species would therefore appear, so far as can be told at present, to be the type form of *A rhodesiensis* and the eastern form is some other variety or species. Careful examination shows many other differences than those mentioned above and the larval characters are quite distinct. Further the pharyngeal characters are so entirely different in the two species (as will be seen from the descriptions and illustrations) that there can be no question of them being merely varietal forms and they are clearly quite distinct species.

The correct name for the species from eastern localities appears to be *A dthali* Patton. *A dthali* was taken by Patton at Dthala, at Sulek and at Nobat Dakim, from which last mentioned locality material was later obtained by Christophers and Khazan Chand. There appears no doubt from Patton's description that he was dealing with this species and it is very unlikely there could be another species with such peculiar features in the same locality. Patton's description of the larva so far as the points given are concerned also tallies, but the characters of the egg as given by Patton are not in accordance with those of the egg of this species as determined by one of us (I. M. P.) from specimens taken at Jandola on the North-West Frontier of India (Waziristan). The figure of the egg given by Patton and reproduced by Edwards (1921, p. 268) has floats with a narrow striated frill not continued over these (i.e., has much the appearance of the egg of *A sergenti*) whereas the egg as determined by us has no floats, but a continuous wide frill.

A full detailed description of the adult characters of *A dthali** has already been given by Christophers and Khazan Chand (1915) and a full description of the larval characters will be shortly published in a *Memoir* by one of us (I. M. P.) dealing comprehensively with the characters of Indian Anopheline larvæ. The hypopygium of *A dthali* has been described and figured (as *A rhodesiensis*) by Christophers (1919) and later (as *A rhodesiensis*) by Kirkpatrick (1925), whilst the pharyngeal characters have been dealt with (as *A rhodesiensis*) by Sinton and Covell (1927) and Barraud and Covell (1928,

* The usual spelling has been *A d'thali* according to the apparently correct way of spelling the name of the locality. But as Patton gives the species name *A dthali* this is more correct as well as more convenient and we have followed this spelling.

1929) Beyond a few special remarks therefore further description here of this species is unnecessary

Regarding *A rhodesiensis*, however, there is no description beyond Theobald's original one in 1901 and a short description by Bedford (1928) Further neither the hypopygium, nor the pharyngeal characters, nor the pleural hairs of the larva, have as yet been described In this case therefore we give a full description of the species (adult and larva) in the Appendix to this paper The egg is not known

Differences between A rhodesiensis *and* A dthali

A rhodesiensis is a moderate sized to small robust, dark mosquito, the pale markings of which are pure white *A dthali* is a smaller, more delicate, brownish mosquito the pale markings of which are not pure white

Head-scales—These are narrow but more or less of ordinary type in *A rhodesiensis*, black over the greater part of the head and white at the top of the vertex the striations extend about 2/3 down the scale* In *A dthali* the scales are markedly linear and rod-like, as much so as in *A aitkeni*, only the tip of the scale is expanded and the striations extend at most a third, usually less, down the scale The scales are uniformly light brownish straw coloured over the whole head area (*Cf* Plate LXIII, figs 1 and 2)

Female palpi—The markings in *A rhodesiensis* are narrow but very distinct and white, the apex is also often pale (not white) In *A dthali* the palps are very inconspicuously banded, often bands are difficult to make out at all, the apex is dark

Mesonotum—This is characteristically shiny and bare in *A dthali* and scales are entirely absent In *A rhodesiensis* the thorax is not particularly smooth and shiny and there is a conspicuous tuft of thin white erect scales in the middle line of the anterior promontory

Wing—The general markings are very similar except that these are more black and white in *A rhodesiensis* In *A rhodesiensis* there is almost always a white spot (line of white scales) near the base of vein 1, whilst in *A dthali* this area is either dark or indefinite The scaling in *A rhodesiensis* is somewhat broader, maximum striation for squame scales about 7-8, as against 5-6 for *A dthali*

Legs—These are much blacker in *A rhodesiensis* with practically no indication whatever of pale markings at any of the joints In *A dthali* the legs are lighter coloured and there is commonly some degree of lighter coloration at the apices of the femora and tibiæ

Abdomen—In *A dthali* the abdomen is commonly very characteristic-blotched with black and lighter markings and the hairs are noticeably light

* In both species the head-scales are unusually long, about half again or twice as long as in most other species

specimen of *A. dthali* (labelled S. Palestine, Shallah, Oct. 1922). Though there is no previous record this species therefore also occurs in Palestine. Covell (1927) gives '*A. rhodesiensis*' as recorded from North-West Frontier Province: Kohat (Sinton, 1917), Khajuri, Sadgi, Murtazza Jatta (Sinton, 1922); Piazi (C. M. B.); Jandola, Kotkai (Clyde), Baluchistan: Quetta, Lora Stream (Browse, 1922). Specimens from all these observers and also from Col. Davys, Quetta, are in the Kasauli collection and are *A. dthali*. *A. dthali* was also taken by one of us (H. M. P.) and adults reared from the egg at Jandola (Waziristan).

Kirkpatrick's records of '*A. rhodesiensis*' from Egypt, it is clear from his descriptions, relate to *A. dthali*. He records this species from Eastern Sinai, inland and about 60 miles S. E. of El Arish, viz., at Kossaima, El Moweilleh, Ain Gedeirat and Ain Kadeis: in all of which localities it was abundant.

The present recorded distribution of *A. dthali* is therefore: South Palestine, Sinai, Upper Mesopotamia, Aden, Muscat, Baluchistan, Waziristan, North-West Frontier Province (India). The distribution is Middle East and may provisionally be classed as **Eastern Mediterranean**.

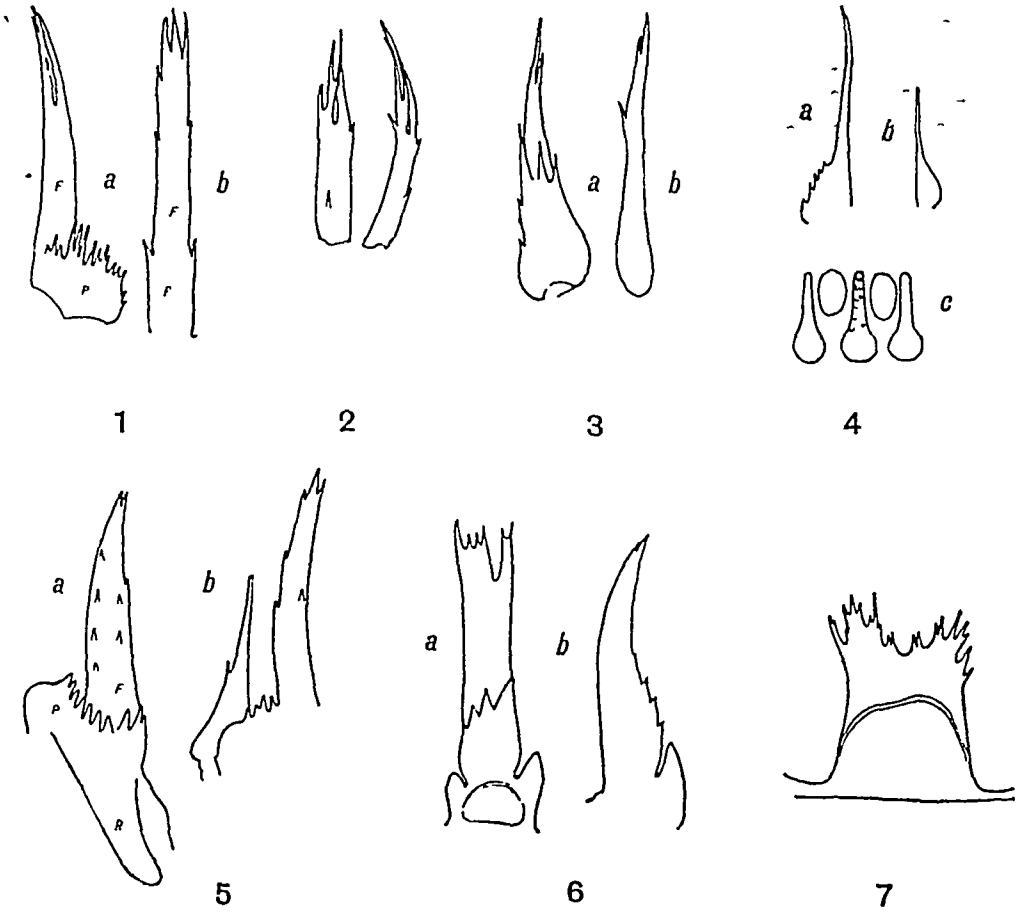
Affinities of A. rhodesiensis Theo.

The pharyngeal armature of this species closely resembles that seen in group *Neomyzomyia*. The characters of the pleural hairs of the larva do not, however, conform with the very marked peculiarities of this group (all hairs simple) and generally resemble the arrangement in group *Myzomyia* but are exceptional in the same manner as *A. sergenti* in having one long mesothoracic hair sparsely feathered. *A. nili* Theo. has a rather similar pharyngeal armature and possibly may not be group *Neomyzomyia* as placed in Barraud and Covell's list. For the present *A. rhodesiensis* must be regarded as a peculiar species in group *Myzomyia*.

A. dthali appears to be a typical member of group *Myzomyia*.

A. (Myzomyia) theileri Edwards.

Larvæ of this species were obtained in a seepage swamp at Mabang in the Protectorate and adults bred out. All the specimens (4 ♀ and 3 ♂) showed the hind tarsus with the first segment entirely dark or very narrowly white at the apex, the second segment with about 1/6 of its length white (in one male it was 1/8 and in another 1/5) and the last three segments all white (Plate LXIII, fig. 4). The costa in all cases had two pale interruptions near the base, an extensive pale area on vein 1 opposite the stem of the anterior fork was present in 4 specimens, the stem of vein 2 and most of the upper branch was continuously pale in 4, largely dark in the others, vein 5 in all had a moderate-sized black spot near the base, vein 6 was mainly dark in the outer half (either a long continuous dark area or two dark spots) in 6 specimens, the remaining specimen having a dark spot only in the middle of the vein. Plate LXIII, fig. 3, gives a female wing taken at random.



TEXT-FIGURE 1

Showing characters of teeth of pharyngeal armature —

1 Isolated 'cone,' *A dthali*, *a* side view, *b* front view, F filament, P pediment

2 Broken off filament *ditto*, anterior and lateral views showing fimbriated extremity

3 Isolated 'rod,' *A dthali*, *a* lateral, *b* antero-posterior view

4 *a* side view of 'cone' of *A theileri*, *b ditto* of 'rod,' *c* usual view seen of pharyngeal armature showing circular bases of origin of rods arising between pediments of the cones which project backwards in a keel-like fashion and give appearance as though of points of the cones In *c* the middle cone pediment shows spines arising along the crest foreshortened and giving 'striated' effect, neither cones nor rods are really seen as these project towards the observer and are out of focus Some modification of the above is commonly shown in Myzomyia, etc, when, as is most usual, the pharyngeal plate is mounted on the flat *Vide* armature of *A dthali*, Plate LXIV, fig 2

5 *a* side view of 'cone' of *A gambiæ*, showing filament (F) and pediment with crest of spines and posterior boss (P), *b* a 'rod' of *ditto* shown in situ behind 'cone', slightly oblique lateral view *Vide* also Plate LXIV, fig 3

6 Pharyngeal tooth of *A rhodesiensis*, showing cusp on either side at base, spinose crown above this and fimbriated filament, *a* anterior, *b* lateral view A single type of tooth only present arising in plane of pharyngeal plate *Vide* also Plate LXIV, fig 1

7 Pharyngeal tooth of *A smithi* Vide also Plate LXIV, fig 4,

All figures are camera lucida drawings to same scale

Recently Edwards (1929) has described three varieties in addition to the type form of this species These may be usefully synopticized in the accompanying schema Where a blank is left the particular variety in question shows the opposite character to that noted at the head of the column, a cross indicating possession of the character

Synopsis of varieties of A theileri *Edw*

	Costa without interruption at base	Wings largely yellow scaled*	Segment 3 of hind tarsus entirely pale†	Segment 1 of hind tarsus rather broadly white at apex (plus base of segment 2)	Segment 1 of hind tarsus also with narrow dark band	Segment 1 of hind tarsus entirely dark
A theileri type	+	+				
var *brohieri*		+				
var *hancocki*			+			+
var *seydeli*				+	+	
var ‡		+	+			±

*ie, in *A theileri* type, wings extensively yellow (large yellow area on vein 1 above stem of upper fork, stem of upper fork entirely yellow and also most of upper branch, dark spot at base of vein 5 small or absent, vein 6 with two or three small dark spots) or in var *brohieri,* wing less extensively yellow (stem of upper fork entirely yellow, but yellow area on vein 1 less extensive, dark area at base of 5 longer, vein 6 mainly dark on apical half) In the other two varieties the wing is described as mainly dark (one or two dark spots on stem of upper fork, vein 5 with long black spot near base, vein 6 mainly or entirely dark in outer half

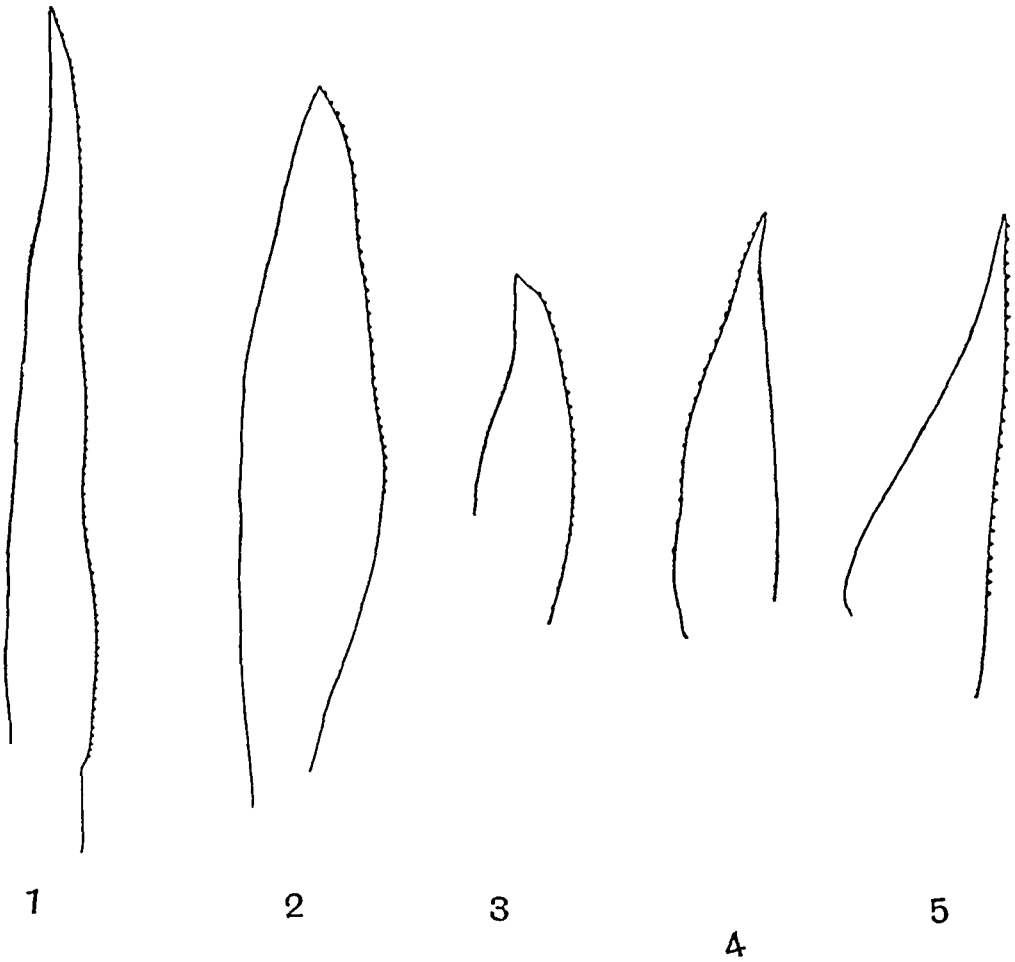
†ie, hind tarsus without dark band, all the last three segments being continuously white

‡ Mabang specimens

The wing characters of our specimens might have fitted in with var *brohieri,* but this is not supported by the tarsal ornamentation On tarsal characters these specimens would come under var *hancocki,* but it is doubtful how far the wings can be distinguished as 'largely dark'* For the present therefore we have recorded these specimens under the species designation only The Mabang specimens, however, differ from the type or Transvaal form as

* Edwards gives under this designation 'one or two dark spots on stem of upper fork, fifth vein with long black spot near base, outer half of sixth vein mainly dark'

described by Theobald (1911), Bedford (1929) and Edwards (1929) in which there is an extra black band on the hind tarsus and no pale interruptions near the base of the costa



TEXT-FIGURE 2

Showing termination of mandible with mandibular teeth of (1) *A rhodesiensis*, (2) *A dthali*, (3) *A theileri*, (4) *A smithii*, and (5) *A gambiæ* All figures drawn to same scale, camera lucida with Zeiss obj 40 and eyepiece 15 and reproduced about half

Adult characters

The species was described by Theobald (1911) under the name of *Pyretophorus albipes* (nec *A albipes* Theo, 1901) (*A theileri* nom nov, Edwards, 1912) and more recently by Bedford (1928) Some of its features are also noted by Evans (1927) and Edwards (1929) The presence of a prosternal hair is noted by the last-mentioned author The female palps are moderately heterodactylous, palpal index 0 4 The anterior pronotal lobes (prothoracic lobes) are devoid of scale tufts The cerci in the female and

coxites in the male are without scales The female hypopygium is of the usual type, insula with 7-8 hairs on each side

Hypopygium

The male hypopygium does not appear to have been described It is of the usual *Myzomyia* type, *harpago* with two large spines, an apical spine about the same length as the organ excluding the club and a spine about 2/3 the length of this lying between it and the club, *phallosome* about ½ the length of coxite excluding leaflets, with about 5 leaflets on each side, the longest about half the length of the organ excluding the leaflets, leaflets with usual fusiform appearance when displayed as usually mounted, but (as commonly the case in Anopheles) somewhat broad, flattened and claw-shaped with some serrations on ventral edge when seen on the flat (Text-figure 3, 3)

Pharyngeal characters

This species is not included in Sinton and Covell's or Barraud and Covell's lists The following are the chief pharyngeal characters

Dorsal papillæ 6, posterior pair widely separated from the others, pigmented area hour-glass shaped

Origin of pharyngeal armature moderately convex Pharyngeal teeth consisting of two rows (rods and cones) without anteriorly projecting roots *Cones* consisting of filament and pediment,* the filament narrow slightly curved thorn-like, smooth and without spicules, pediment narrow keel-like, set with numerous fine sharp spines along crest, crest rising gradually to base of filament *Rods* somewhat shorter than cones, rising from expanded oval basal areas, with bulbous base and tapering extremity, smooth, without spicules or bifurcations (*vide* Text-figure 1, 4)

Pharyngeal ridges numerous, short, each with 3-4 moderately developed spines

Mandibles and maxillæ

Mandibles with about 24 teeth (Text-figure 2, 3), maxillæ with 11 teeth

Larval characters

A number of the larval characters have been described by Blacklock and Evans (1925) A full description is, however, given in the appendix to this paper, including description of the, as yet undescribed, pleural hairs which are of group *Myzomyia* character The pupa has been described by Ingram and de Meillon (1929)

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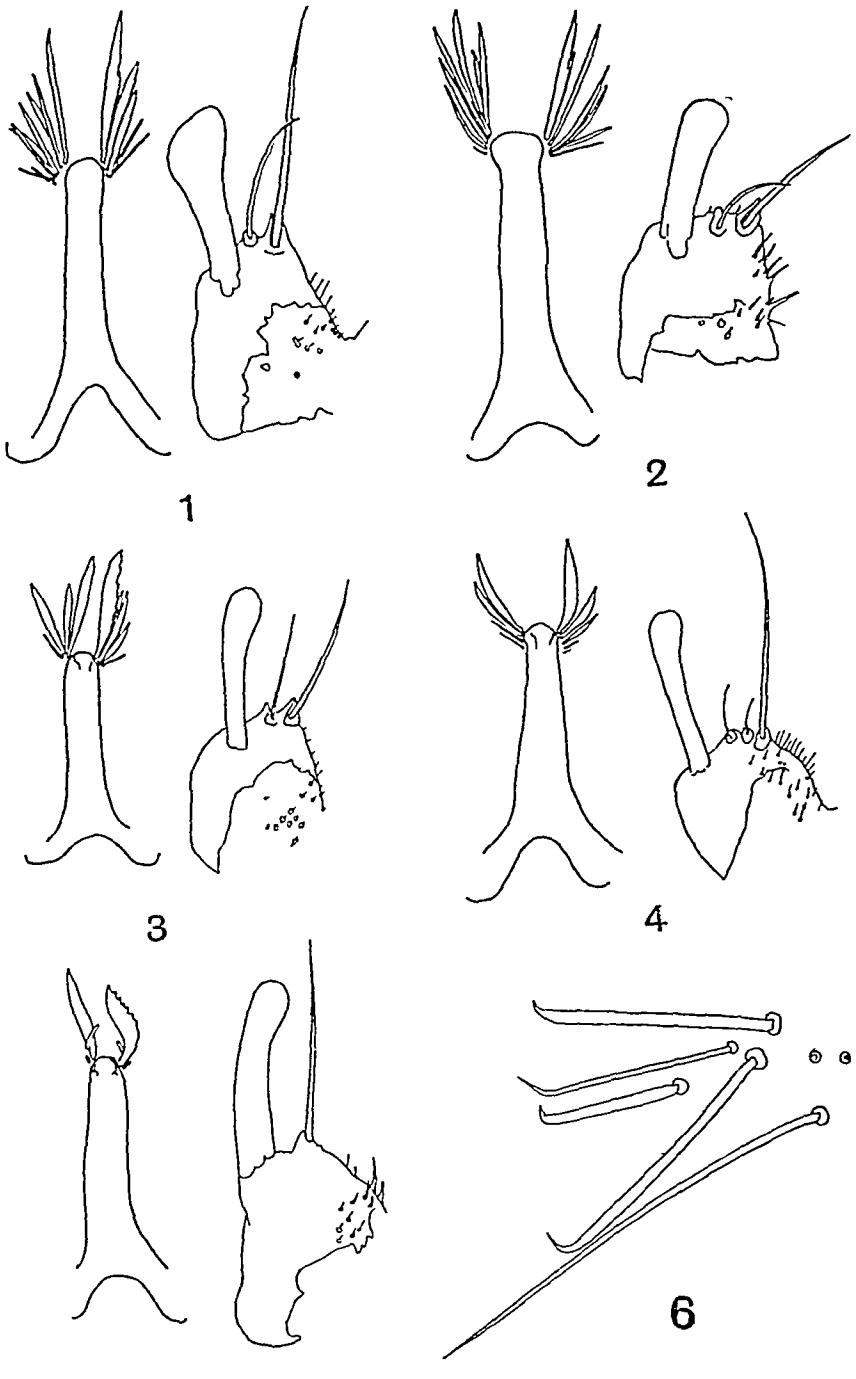
* A further description of the structure of the pharyngeal teeth in Anopheles will shortly be given by one of us (S R C) The 'cones' usually have a backwardly projecting basal flange or rudder-like basal extension armed with spines along its crest (*pediment*), the apical prolongation of the tooth is the *filament* When viewed as the buccal cavity is ordinarily mounted the filaments in many species lie more or less directed towards the observer and are only seen in optical section on focusing What may appear like the filament is the crest of the pediment looked down upon The condition will be clear from the drawings and explanations of Text-figure 1



TEXT-FIGURE 3

Showing phallosome and harpago of (1) *A rhodesiensis*, (2) *A dthali*, (3) *A theileri*, (4) *A gambiæ*, (5) *A smithii*, also (6) parabasal spines of *A smithii* with short inner and thin middle hair of upper row The two hair bases on the right hand side are outliers of the vestitural hairs on the outer aspect of the coxite
Camera lucida drawings to same scale

Distribution

This is typically Ethiopian [Transvaal, Natal (Zululand), Belgian Congo, Uganda, Nigeria, Gold Coast, Sierra Leone]

Affinities of A. theileri

Though the hind tarsi are tipped with white as is very usual in the *Neocellia* group, a single propleural hair is present, and the larval characters as shown by the pleural hairs are those of group *Myzomyia* [*vide* ref. by Edwards (1929) to private communication by one of us on this subject]

A. (Myzomyia) gambiæ Giles

This species was found breeding in various places around Freetown

Adult characters

Many descriptions of this species exist, the most detailed description of the adult characters is that by Christophers and Khazan Chand (1915). The female palpi are rather orthodactylous. The anterior pronotal lobes carry scale tufts.

Hypopygium

The male hypopygium has been described and figured by Christophers (1915). A further drawing of the harpago and phallosome is given in Text-figure 3, *4*.

Pharyngeal characters

These have been described by Barraud and Covell (1928). The following is a more detailed description of the pharyngeal teeth.

Pharyngeal teeth forming two rows (rods and cones), the rods shorter than the cones. *Cones* with filament and pedment, filament large, flat, bluntly tapering, armed laterally and on surfaces with spicules, pediments terminating posteriorly in a rounded boss, the spines of the crest passing well round base of filament anteriorly. *Rods* conical, rising from broad circular base with one or two spicules, the apical portion narrow rod-like, the whole a little more than half the length of cone. From the base of the cones there extend deeply into the substance of the hard palate apparently hollow bullous extensions (*vide* Plate LXIII, fig. 3, and Text-figure 1, *5*).

Mandibles and maxillæ

Mandibles with about 30 teeth (Text-figure 2, *5*), maxillæ with about 10 teeth.

Larval characters

These have been described shortly by Hill and Haydon (1907) and a number of the larval features have recently been figured by Blacklock and Evans (1926) and Evans (1927) as well as various references by other authors (*vide* bibliography at end of paper). A full description of the larva is, however, given in the appendix which includes for the first time the characters of the important pleural hairs. The pupa has been described by Bacot (1916).

In their arrangement and branching the pleural hairs of *A gambiæ* resemble those of group *Myzomyia* i e, prothoracic, 2 long simple and 1 long feathered, mesothoracic, 2 long simple, metathoracic, 1 long simple, 1 long feathered But these hairs show a marked *Pseudomyzomyia* character in the presence of a large sharp spine on the basal tubercle of the mesothoracic hairs arising from the inner side (Plate LXV, fig 4) The larval characters further resemble those of *Pseudomyzomyia* in that the various hairs on the body have comparatively few branches, and they also resemble *Pseudomyzomyia* and differ from *Myzomyia* in respect to the very small size of the tergal plates and the absence of a differentiated metathoracic palmate hair

The egg of this species has been described by Annet, Dutton and Elliot (1900), and, under the name of *A arabiensis*, by Patton (1905) Patton's drawing is reproduced by Edwards (1921) It is of a type somewhat resembling the egg of *A culicifacies*, with a narrow upper deck and floats not quite touching the frill

Distribution

A gambiæ has been recorded* from numerous localities throughout tropical and south Africa as far south as Isipingo (south of Durban, Natal, 30° S), Vryburg (British Bechuanaland, 27° S) and Franzfontein (South-West Africa) and as far north as Gara Sabo (Mauritania), Dagana (northern boundary of Senegal, 17° N), Katagum (Northern Nigeria, 12° N) and Khartoum, 16° N The species also occurs in Madagascar, Mauritius and Reunion It occurs also in the Aden Hinterland (S W Arabia, 13° N) but has not yet been recorded from Egypt or S E Arabia (Muscat) The known area of distribution may therefore be roughly given as Africa and larger Islands and including the S W corner of Arabia between 30° S and 17° N

A specimen, however, of *A gambiæ* is in the Paris Museum with locality Algeria (*vide* Edwards, 1921, p 278, Seguy, 1924, p 163) and the species has recently been recorded from Asia Minor (Hakki, Rev Med et Hyg Trop, 19, p 8, ref Rev App Ent, 15, p 80, 1927), from Rio Grande do Norte, Brazil† (da Costa Lima, Science, 71, p 435, 1930, ref Rev App Ent, 18, p 150, 1930) and a single female from northern Greece (Seguy, Encyc, Ent, Ser B, Dipt, 5, p 177, 1929, ref Rev App Ent, 18, p 161, 1930) Presumably these are recent importations and not in the normal area of distribution

Affinities of A gambiæ *Giles*

The adult characters, especially the presence of a prothoracic tuft, are not suggestive of group *Myzomia*, and the presence of a prosternal hair appears to exclude *Neocellia*, on the whole the adult characters conform most closely to *Pseudomyzomyia* in which group this species was placed by Christophers (1924) This location receives strong confirmation from the pharyngeal

* *Vide* Bedford (1928) and Kumm (1929)

† This is about the nearest part of South America to West Africa

characters and though the larval characters are ambiguous they also show a number of *Pseudomyzomyia* affinities

A (Myzomyia) funestus Giles

It was disappointing that only a single specimen of this species, one of the reasons for visiting Sierra Leone, was obtained, most of the likely breeding-places being in spate due to heavy rain Discussion of the species with especial relation to allied Indian forms will therefore be more suitably dealt with elsewhere, it is hoped shortly, by us

A (Myzomyia) freetownensis Evans

A single specimen only of a species provisionally identified as *A marshalli* Theo was taken as a pupa in a pool by the side of a rocky river in flood (Kissy bridge) On examination it showed all the characters of var *freetownensis* Evans As the larval characters have been shown to be quite distinct by Evans from those of *A marshalli* and as the adult characters are different it would seem that this should be regarded as a species rather than a variety

The accompanying synoptic abstract of characters of the different *A marshalli*-like forms recognized up to the present, based on a study of the literature and also examination of certain of the type specimens by one of us, may be useful A bibliography of the different forms is given at the end of this paper

A (Myzomyia) smithii Theo 1905

Numerous larvæ of this species were obtained from a pool under an overhanging rock on Nicol's Brook, one of the breeding-places referred to by Blacklock and Evans (1906)

Adult characters

The female has been described by Theobald (1905, 1907) and the male by the same author as *Feltinella palpidopalpi* in 1907 Remarks on the species are given by Christophers (1924) and Evans (1926, 1927) the last-mentioned author noting the great difference in the degree of wing ornamentation in the male and female The following are additional or important characters

Antenna of female with torus devoid of scales and (as noted by Theobald) segment 2 with outstanding scales (dark) Palpi of female rather cylindrical and thick towards tip, the segments commencing with the rudimentary basal segment measuring 5, 30, 41, 15 and 9 per cent of the whole organ respectively, palpal index 0 59 Palpi of male extensively golden yellow on both segments of club and at 2-3 (i e, as in highly ornamented species, not as one might expect in a species primarily poorly ornamented) Head-scales of usual type with about 13 striations

Anterior pronotal lobes (prothoracic lobes) with marked scale tuft (as noted by Theobald), propleural hair 1 long, stout Mesonotum dark with tomentose effects, with chætæ only except anteriorly where there is a median tuft on anterior promontory of pale narrow erect scales and some dark erect

Synopsis of species and varieties related to A marshalli

	White rings at bases of last four hind tarsal joints (apical and basal banding), dark area at base vein 5 more than 1/4 stem	Tarsal banding broad on all legs, on front legs may be 1/4 some segments A fringe spot basal to vein 6	Tarsi entirely unbanded, pre-apical dark spot on costa short	Dark spot on fringe at apex absent, apical dark spot on costa minute, pale markings on costa and vein 1 very yellow	Mesonotal scaling on anterior half distinctly broad	Mesonotal scaling on anterior half broad but that on posterior half to 2/3 narrow	Wing scales broader than type *A marshalli*	Pale area at apex of costa extensive, equal to or greater than dark	Preapical dark spot on costa with interruption on vein 1	No fringe spot at vein 6	Base of costa uninterruptedly dark—not two dark accessory spots	Dark band between pale apical bands on female palps only somewhat shorter than pale area
A domicolus	+						+			+		
A aurem		+			+				+	+	+	
A fieldwensis			+									
A flavicosta				+				+	?		?	
var *hargreavesi*					+		+	+		?		
var *moucheti*					+	+	+					
A pitchfordi		(a)						+				+
A marshalli									+			+

(a) Front tarsi in this species rather broadly banded
Where a space is left blank the species does not show this particular character

scales laterally, chætæ dark and well developed notably anteriorly on the lateral portions of anterior promontory Spiracular hairs 3, prealar hairs absent, sternopleural, row of 3 in upper and 5 in lower group, upper mesepimeral hairs 2

Wing with base to subcostal junction 0 62, anterior forked cell 0 26 and posterior forked cell 0 14 of whole organ respectively, forked cell index 1 8 Scales of wing in general character oval and entire, truncated scales limited to basal part of wing and even then not conspicuous Scales broad, maximum striation 9-10

Coxæ devoid of scales, first trochanters with scales and hairs, 2 and 3 with hairs only Legs uniformly dark scaled with fairly distinct knee spots at ends of femora and tibiæ Male ungues of usual type

Abdomen with dark hairs Coxites in male and cerci in female with numerous scales Female hypopygium of usual type, the insula with about 6 hairs on each side

Hypopygium

The hypopygium is referred to by Christophers (1924) and figured by Evans (1926) It is of *Myzomyia* type in general *Parabasal spines* 5, of the most anterior row of three hairs the inner is very short and stout arising from a more or less distinct lobe-like eminence on the coxite, the middle hair is slender (depicted so in Miss Evan's drawing) and the outer hair of usual type, there are no small accessory hairs on inner aspect of coxite but one or two vestitural hairs on the outer aspect come near the group and if turned inwards may appear almost as additional hairs *Harpago* of usual *Myzomia* type about $\frac{1}{4}$ length of coxite, with club and single large apical hair slightly longer than club, some rather well developed small spines on inner aspect *Phallosome* moderately straight, about $\frac{1}{2}$ length of coxite, carrying 3 leaflets on each side, one large about $\frac{1}{4}$ length whole organ, the other about half length of this and a minute third spicule, the large leaflets broad, flat, claw-shaped, toothed on ventral edge (Text-figure 3, *5*)

Pharyngeal characters

Dorsal papillæ 6, posterior pair widely separated from others, pigmented area hour-glass shaped

Origin of pharyngeal armature concave Pharyngeal teeth 5, 3 very large, broad, short, projecting in same line as pharyngeal plate, with conspicuous semilunar bulla at base and truncated, spiny or irregular fimbriated extremity, one smaller less developed tooth on each side of these Teeth are separated by wide intervals forming a single row without any indication of rod and cone effect, etc, and are of the *Neomyzomyia* group type of Sinton and Covell (1927) and Bairaud and Covell (1928, 1929) (*vide* Plate LXIV, fig 4, and Text-figure 1, *7*)

Mandibles and maxillæ

Mandibles with about 25 teeth (Text-figure 2, *4*), maxillæ with 16 teeth, 12 of these at least large and easily counted

Larval characters

A number of the characters have been described by Blacklock and Evans (1926), but a systematic description of the larva is given in the appendix and the pleural hairs described All are simple as in subgenus *Anopheles,* i e, this species on larval characters (excluding the possibility of subgenus *Anopheles* as clearly shown by the hypopygial characters, etc) belongs to group *Neomyzomyia* to which the pharyngeal characters also conform

Distribution

Up to the present noted only from Sierra Leone

Affinities of A smithi *Theo*

A smithi is clearly another African *Neomyzomyia,* the other African members of which group are all Transvaal or East African forms This is the most westerly outher of this group which except in the Australasian and eastern Oriental region may be considered, compared to other groups, as approaching a palæogenic distribution

Summary

1 *A rhodesiensis* and *A dthali* The species from Arabia, N W India, etc, up to now recorded as *A rhodesiensis* Theo is a distinct species *A dthali* Patton, the distribution of the two species being respectively Ethiopian and Eastern Mediterranean Points of distinction between the two species are given and the distribution of each dealt with In the appendix is a complete description of *A rhodesiensis* Theo, including hypopygial and pharyngeal characters and pleural hairs of the larva, none of which characters have as yet been described by any author, all previous descriptions relating to *A dthali* Patton *A dthali* is typical group *Myzomyia,* but the pharyngeal and larval characters of *A rhodesiensis* are peculiar and related respectively to the aberrant species *A nili* and *A sergenti*

2 *A theileri*—The specimens taken in the Protectorate do not conform to any of the varietal forms named by Edwards, a synopsis of which is given The, as yet undescribed, hypopygial and pharyngeal characters of this species are now given and in the appendix is a complete description of the larva including the, as yet undescribed, pleural hairs of this species *A theileri* in all but the high *Neocellia*-like ornamentation conforms to group *Myzomyia*

3 *A gambiæ*—The pharyngeal characters and pleural hairs of the larva are described, the former with further information regarding the actual structure of the pharyngeal teeth The pharyngeal characters are those of

group *Pseudomyzomyia* with which a number of the larval characters agree, but the actual branching of the pleural hairs is that seen in group *Myzomyia*

4 *A freetownensis*—A synopsis of *A marshallii*-like forms is given largely based on the work of Evans but also on observations of types, etc, by one of the authors On the data available, notably the larval characters, var *freetownensis* is given as a species

5 *A funestus*—Discussion of this species is postponed

6 *A smithii*—Some new points are given about this species including the pharyngeal characters and description of the pleural hairs of the larva The pharyngeal and larval characters clearly show this species to be a further African *Neomyzomyia* a group which shows a comparatively palæogenic type of distribution

7 A bibliography for each of the species dealt with is given

8 In the appendix are given descriptions of *A rhodesiensis* (adult and larva) and of the full-grown larva of *A theileri* Edw, *A gambiæ* Giles and *A smithii* Theo, including in each case a description of the pleural hairs

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APPENDIX

A (Myzomyia) rhodesiensis THEOBALD

Description of adult

Diagnostic points

(1) Costa spotted but the wing-field without any pale markings
(2) Head-scales black over the back of the occiput, a white spot on vertex
(3) Mesonotum with some white scales forming a tuft on the anterior promontory

Detailed description

Female

A moderate-sized Anopheline length of wing 2·7–3·5 mm General appearance dark, resembling *A funestus*

Head-scales of ordinary Anopheline type, unusually long (0·1 mm), narrow and linear especially towards the sides of the head, but in centre more expanded, with about 12 easily counted well-marked striations, some reaching two-thirds or more down the length of the scale Scales confined to area above neck, black over greater part of scaled area but white on upper part of vertex, continued forward through some small modified scales to a fairly developed frontal tuft composed of the usual white elongated scales and chætæ *Antenna* including the tora without scales *Palpi* 1·7 of thorax, 0·65 of wing, the five segments commencing with the rudimentary basal segment measuring 5, 31, 37, 21 and 6 per cent respectively of the whole organ Palpal index 0·3 Narrow but distinct white bands, mainly apical, at 2–3 and 3–4, no pale band at 4–5, the apex usually somewhat pale but not white or sharply demarcated as with other bands No scales on rudimentary first segment, the rest of the organ covered with appressed scales showing not above 7 striations, the whole organ narrow, smooth and cylindrical from base *Labium* dark scaled except labella *Clypeus* without scales

Pronotal lobes with chætæ only *Prosternal hairs*, one, short, stout *Mesonotum* not specially shiny, covered rather regularly with dark and some thin lighter coloured chætæ having the usual arrangement, no scales of any kind except anteriorly in the middle line on the anterior promontory where there is a cluster of white, narrow upstanding scales, somewhat variable in extent and in breadth of component scales No lateral scale tufts *Pleuræ* devoid of scales Pre-alar hairs about 2, spiracular 2–3, upper mesepimeral about 6, upper and lower sternopleural not more in each case than 3 or 4 No spiracular hairs

Wings average length in a good specimen 3·3 mm, about 2·7 of thorax Base to subcostal junction 0·55, anterior forked cell 0·28, posterior forked cell 0·16 of the length of the whole wing Forked cell index 1·75

Normally four moderate sized, about equal, pale areas, about one-quarter extent of corresponding dark areas, on costa and first longitudinal, viz, apical, subapical, subcostal and sector Costa unbrokenly dark from sector to base of wing (about 1/3 of length of wing), the first longitudinal, however, in this position with a white spot more or less extensive but usually occupying the middle third of this portion of the vein, the inner dark third being the remigium Subcosta marked as costa, joining costa at inner end of subcostal spot The remainder of wing including the fringe and border scales is quite devoid of pale markings, but may show a few pale scales in some cases at the cross-veins (Evans, 1927) In all our specimens the wing-field was uniformly dark The wing fringe is without pale markings

following upon the apical pale spot, but there is usually a darker tache at the apex at 2·2 to 3·1. Scaling of the wing is moderately narrow, maximum striation for squames 7–8, for plume scales about 6. The plume scales on the basal portion of vein 1 are noticeable for their length.

Coxæ devoid of scales. All *trochanters* with hairs and scales. Legs uniformly dark to bases with scarcely a trace of lighter marking at apices of femora and tibiæ. *Tarsus* unbanded. *Male ungues* of normal character.

Abdomen with segments entirely devoid of scales. *Cerci* with hairs only. *Female hypopygium* of usual Anopheline character, insula with about 12 hairs on each side.

Male.

In general as the female. *Palpi* 1·7 of thorax, 0·6 of wing, the five segments 6·25, 3·7, 1·8 and 1·3 per cent. respectively of the whole organ. Uniformly dark scaled except at the tip which is indistinctly pale. Apical segments (club) narrow, fusiform. *Wing* with usual characters of male wing, base to subcostal junction 0·58, anterior forked cell 0·23, posterior forked cell 0·13 of the length of the whole wing. *Male ungues* of usual type. *Abdomen* without scales. *Coxites* with some scales towards the base externally.

Hypopygial characters (Text-figure 3, *I*).

Parabasal spines 5 in number with the usual subgenus *Myzomyia* character and arrangement, i.e., 3 spines of about the same length and rather short, not very modified with tapering points, arising in a line somewhat obliquely across the base of the coxite, the inner rather more hooked than the others, a much longer, stouter and blade-like spine arising close to, distal and opposite the gap between the two outer spines of the first row, its end broad and rather hooked and a fifth much the longest but thinner and resembling a vestitural hair of the coxite arising somewhat distal and external to the last-mentioned hair. About 12 small accessory hairs distal to spines on inner aspect of coxite. *Harpago* unilobar with the usual club arising from dorsal border. A stout spine, about half again as long as the club arising from the apex of the organ and a smaller thinner hair about half its length arising between the larger spine and the club. The inner aspect of the harpago without any conspicuous development of small accessory hairs. *Phallosome* moderately straight and slender, when measured from roots to apex and excluding leaflets about half the length of the coxite, carrying about 5 leaflets on each side, the longest about half the length of the phallosome measured as above, the leaflets stout seen as fusiform rods or if on flat as slightly curved flattened, claw-shaped blades with some serrations on ventral edge. Ninth tergite with triangular lateral portions without projecting processes.

Pharyngeal characters (Plate LXIV, fig. 1, and Text-figure 1, *C*).

Dorsal papillæ 6, posterior pair widely separated from others, pigmented area hour-glass shaped, lateral flanges of moderate size with two or three small indefinite teeth.

Line of origin of pharyngeal armature only moderately convex. Pharyngeal teeth 8, separated by intervals, rising at edge of hard palate, basal bulla large, clear, distinct, not extending into hard palate. Filaments tapering flat, with fimbriated ends, on either side at base a short, stout, blunt, tooth-like projection, that on outer side the larger except in case of middle tooth where those on either side are equally developed. Above this on posterior aspect of filament is a circlet of sharp spines. Bucco-pharyngeal ridges without, or with very short hairs.

Mandibles and maxillæ.

Mandibles with about 80 teeth (Text-figure 2,), maxillæ with about 11 teeth.

Description of the full-grown larva.

Head.

The *head* is golden brown in colour. The usual spots are well developed and surrounded by a dark brown cloud which is fairly extensive merging the various spots into one another

In some specimens the posterior region of the head is very dark with a large dark brown spot in front of it covering most of the dorsum of the head *Anterior clypeal hairs* with both pairs finely frayed having 4 to 7 very slender minute branches often difficult to see The inner anterior clypeal hairs are comparatively slender, av length 0·23 mm , outer anterior hairs, av length 0·12 mm , av clypeal hair index 0·53 *Posterior clypeal hairs* always simple and a little longer than the outer anterior, av length 0·13 These lie external to inner anterior hairs, their distal end reaching a little beyond the bases of these *Inner sutural* about as long as outer anterior clypeal and simple, *outer sutural* somewhat shorter, with 3–5 branches

Antenna throughout brown in colour, bearing on inner surface of basal half a number of conspicuous spinous processes *Antennal hair* very short, simple, arising from the dorso-external surface at basal third of antenna The transparent cone-shaped piece at tip of antenna a trifle shorter than the small finger-shaped piece The *terminal hair* splits about its middle into 3–5 branches *Mandibles* with the pair of sensory hairs on the ventro-external surface minute and simple, arising far apart from each other *Maxillary palp* with the cone-shaped appendage at the tip about one and a half times as long as the single finger-shaped appendage which is a little shorter than the paired finger-shaped pieces *Mentum* fairly broad and bearing on each side of the median tooth a row of four teeth, three anterior ones of which are equidistant from each other whilst the fourth is placed a little further back The anteriormost tooth in each row has a somewhat rounded end

Thorax

Dorsal submedian prothoracic hairs with the inner and middle hairs arising from well-defined chitinized basal tubercles, both the hairs feathered, the inner with 16–24, the middle with 13–19 lateral branches, the outer hair very short and simple *Metathoracic palmate hair* fairly well developed, with 10–16 long lanceolate leaflets without a differentiated filament

Pleural hairs with the arrangement of long hairs as in group *Myzomyia* (prothoracic 2 simple, 1 feathered, mesothoracic 2 simple, metathoracic 1 feathered, 1 simple) except that one mesothoracic hair instead of being simple is sparsely feathered (as in *A sergentii*) Dorsal anterior prothoracic long feathered, the ventral long, simple, dorsal posterior prothoracic one-third length of the ventral anterior and bearing 4 lateral branches, the ventral long, simple Dorsal anterior mesothoracic long sparsely feathered, ventral long, simple, dorsal posterior minute, simple the ventral short, slender, splitting distally into 2 branches Dorsal anterior metathoracic long and sparsely feathered, the ventral long and simple, dorsal posterior minute and simple, the ventral short and slender splitting distally into 2 or 3 branches The chitinous tubercles from which the pleural hairs arise are of moderate size except the one on the mesothorax which is rather small The flattened projection between the pairs of hairs on the prothorax is produced into a pointed spine, those on the other two segments poorly developed

Abdomen

Hair No 1 is transformed into a palmate hair on segments I–VII, that on segment I is not so well developed as on the segments following and has only 8–11 leaflets with the filaments poorly developed The other palmate hairs are well developed and bear 15–17 leaflets on segments II and VII 17–20 on segments III–V and 16–19 on segment VI The blades of the leaflets are more or less uniformly coloured except in the distal third in which they are much lighter The filament of the leaflet is differentiated, but the serrations at the shoulder are not very deep and in some cases very few in number, the filament consequently varying very greatly in its width at the base The average length of the blade is 0·065 mm and that of the filament 0·024 mm , average ratio between length of blade and filament 0·37

Lateral hair long on segments IV–VI with 5 or 6 branches on segment IV and with 4 or 5 on segments V and VI, on segment VII very short with 4–5 branches *Post-spiracular*

SHOWING DETAILED CHÆTOTAXY OF THE LARVÆ OF **A. rhodesiensis** THEO., UP TO ABDOMINAL SEGMENT VIII

Head

Hair number	2	3	4	5–7	8	9	10	11	12	13	14	15	18	20
	f	*f*	1	*F*	1	3–5	1	3–5	*LP*	1–6	1–2	3–5	8–10	1–6

Body

Segments of thorax and abdomen	Hair number	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	I	15–24	13–19	1	*F*	*F*	1	*F*	*F*	*F*	1	1	1	6–7	6–7
	II	*F*	1	1	1	3	1	3	*F*	*F*	1	1	2	6–8	6–7
	III	10–16	3	1	3	*F*	2	*F*	*F*	*F*	1	*m*	2–3	4	
1		8–11	3	1	6	3–5	*F*	*F*	×	5–6	2	1	1	6–7	
2		15–17	8–9	5–6	1	4–5	*F*	*F*	2	7–9	2	3	3	5–8	
3		17–20	4–6	3–5	1	6–7	*F*	5	2	8–9	3	3	1	5–8	
4		17–20	1	3	3	4–5	5–6	3	2	6–7	3	3	1	5–7	
5		17–20	1	3	1	6–8	5	1	2	7–8	3	3	3	4–5	
6		16–19	1	1	1	9	1	3	1	8	3	1	1	6	
7		15–17	7	3	1	9	4	6	1	1	7	2	3	1	
8		1	1	1	2	2	7	2	12	10–12	3	×	×	6	

Note—The numbers indicate branches, *F* = feathered, *f* = frayed, *LP* = very long, pinnate, *m* = minute, × = hair absent

hair long, bearing 6–9 long lateral branches The *saddle hair* almost invariably split into two in its distal third, *outer submedian caudal hair* with 6 or 7 long stout branches, the distal ends of which are curved to form hooks, *inner submedian caudal hair* also with some branches stout and curved distally The ends of some of the branches of the ventral row of hairs also slightly curved

Tergal plates moderately large, their length 0·23 mm, their width nearly one-third of this The small median plate of the usual size The pair of small oval plates found in most of the larvæ belonging to group *Myzomyia* are absent in this species

Spiracular apparatus with the chitinization along the posterior border of the spiracular openings poorly developed, but the median plate of the scoop fairly broad anteriorly so that its antero-lateral border touches the spiracular openings The pigmentation of the plate is as in *A sergenti* larva *Pecten* with 5 long and 9–11 short spinous projections all of which have conspicuous serrations along their dorso-lateral borders

The ventral surface of the body, particularly that of the posterior segments, bears irregular rows of minute setæ easily visible under a moderate magnification

The accompanying chart gives the details of branching, etc, of all the hairs of the larva up to abdominal segment VIII A glance at such chart compared with those of other larvæ will indicate outstanding differences

A (Myzomyia) theileri Edwards

Description of the full-grown larva

Head

The *head* is brownish yellow in colour, the usual dark spots well defined, a diffuse dark brown spot connects the anterior median spot with the two lateral oval spots *Inner anterior clypeal hairs* arising far apart, the distance between their origin about twice or a little less than twice that between the bases of the inner and outer anterior hairs of one side, stout and simple, their length about 0·188 mm and about 1/3 that of the fronto-clypeus *Outer anterior clypeal hairs* stout and simple, length about 0·01 mm, clypeal hair index 0·5 *Posterior clypeal hairs* arising a little external to the inner anterior, their distal end not reaching the bases of the latter, slender, simple, length about half that of outer anterior clypeal hairs or 0·48 mm *Frontal hairs* normal (feathered), the distal ends of the distal pair reaching to the base of the anterior hairs, that of the middle pair, which is only very slightly longer than the outer, extending to base of posterior clypeal hairs *Inner sutural hair* a little longer than the posterior clypeals, slender, simple, *outer sutural* of about the same length, split into 3 branches *Subantennal hair* stout and profusely feathered

Antenna more or less uniformly pigmented, a little less than half the fronto-clypeus, fairly stout, its length 6–7 times its breadth near the base, inner and ventral surfaces with a large number of conspicuous short spinous projections Antennal hair short and simple, arising from the dorso-external surface between 1/3 and 1/5 length of antenna from the base The transparent cone-shaped piece is much shorter than the finger-shaped piece, which is comparatively long in this species The terminal hair splits about its middle into 4–5 branches *Maxillary palp* with the cone-shaped appendage at the tip of the palp about twice the length of the finger-shaped appendage and with simple distal end *Mentum* with 7 teeth Of the row of three teeth on each side of the median one the anteriormost is the smallest and has a rounded end In one specimen there was a small tooth placed at the end of the row on one side

Thorax

Submedian prothoracic hairs with the inner as well as the middle hair stout, the inner, which is a little shorter than the middle, with 22–25 closely set lateral branches, the middle with about 11 Both hairs arise from well-developed dark brown basal tubercles which may be partially fused with each other or separate The outer hair is short and simple

SHOWING DETAILED CHÆTOTAXY OF THE LARVÆ OF **A. theileri** EDW., UP TO ABDOMINAL SEGMENT VIII

Head

Hair number	2	3	4	5–7	8	9	10	11	12	13	14	15	18	20
	1	1	1	*F*	2–3	3	1	1–5	*LP*	3	2	1	1–6	5–6

Body

	Hair number	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Segments of thorax and abdomen	I	22–25	11	1	*F*	*F*	1	*F*	*f*	*F*	1	1–3	1	5–7	5–6
	II	*F*	1–2	1	1	4–5	3–4	5–6	*f*	1	1	*m*	1	9	7
	III	18–22	4	3	1	*F*	3	*F*	*f*	*F*	1	*m*	3–4	3–4	
	1	12–13	3	1	5–6	7	*F*	*f*	×	9	4	4–5	5	7–8	
	2	14–17	8	6–7	1	8	*F*	*f*	2–3	13–14	3–6	5	5	11–12	
	3	17–19	6–8	3–5	1	7–9	*F*	7–8	3	12–14	3–5	4–5	6	14–15	
	4	17–19	1	3	3–4	8	8*F*	7–9	3	16–18	3	1	5–6	14	
	5	17–19	1	3–5	2–3	9–10	8–9*f*	7–8	3	10–13	4–5	3–4	3–5	8	
	6	17–19	1	1	1	12	9*F*	5–6	3	13	3–4	3–4	4–7	12	
	7	14–17	3–5	4–5	2–3	11–14	3–4	10	5	8–9	9–10	3	5	5–6	
	8	1	1	1	2	2	9–10	3–5	11–13	5–13	5–8	×	×	9	

Note—The numbers indicate branches, *F* = feathered, *f* = frayed, *LP* = very long, pinnate, *m* = minute, × = hair absent

Metathoracic palmate hair well developed, with 18–22 lanceolate leaflets arranged in a whorl, the filaments not differentiated

Pleural hairs with the arrangement of long hairs as in group *Myzomyia* (i e, pro 2 simple, 1 feathered, meso- 2 simple, meta- 1 feathered, 1 simple) *Dorsal anterior* prothoracic long and feathered, ventral long, simple, dorsal posterior short with 3–5 lateral branches, ventral long, simple Dorsal and ventral anterior mesothoracic long, simple, dorsal posterior minute, simple, the ventral short, slender and split distally into 4 branches Dorsal anterior metathoracic long and feathered the ventral long and simple, dorsal posterior minute, simple, the ventral short and split distally into 3–4 branches The chitinous tubercles from which the pleural hairs arise are moderately large, the flattened projections between the pairs of hairs on the meso- and meta-thorax are pronounced but rounded distally

Abdomen

Hair No 1 forms well-developed palmate hairs on segments I–VII, though that on segment I is not so well developed as on segments following and has only 11–13 narrow leaflets with poorly-developed filaments The other palmate hairs with 14–17 leaflets on segments II and VII, 17–19 on segments III–VI The basal half of the leaflets is somewhat uniformly coloured while the distal half is much lighter The filament is differentiated, but the indentations at the base are not very deep and extend along the edge so that the filament is very broad at the base Av length of blade of leaflet from mid-abdominal segment 0·05 mm , that of filament 0·02 mm (including the very broad basal portion) The part of the filament free from lateral indentations is very short *Lateral hairs* on segments IV–VI long and bearing 8–9 lateral branches, somewhat resembling a sparsely branched feathered hair, the corresponding hair on segment VII is very short and splits distally into 3–4 branches *Post-spiracular hair* long, with 8–9 lateral branches *Saddle hair*, unlike that of many species, is branched in its distal half bearing 6–7 branches *Outer submedian caudal hair* with 7 stout long branches the ends of which are curved to form hooks, some of the distal branches of the inner caudal hairs are also slightly curved at the end and form poorly developed hooks

Anterior tergal plates are fairly large and the rounded median plate of the usual size, the paired oval plates are absent *Pecten* with 4–5 long and 7–9 short spinous projections all of which are finely serrated along the dorso-lateral border of their basal half

The accompanying chart gives the details of branching, etc of all the hairs of the larva up to abdominal segment VIII

A (Myzomyia) gambiæ GILES

Description of the full-grown larva

Head

The *head* is brownish yellow with the usual dark spots In some specimens the spots are poorly developed one or two only being present, in others all the spots are well defined and dark brown with a horizontal bar-like cloud surrounding the anterior median spot and extending along the bases of the frontal hairs, another large diffuse spot being present in front of these hairs *Anterior clypeal hairs* unusually long, with the inner bearing fine lateral branches on their distal 2/3, the outer simple or bearing lateral branches like the inner The inner anterior clypeal arising with their bases wide apart, the distance between these about twice or more than twice that between the bases of the inner and outer anterior hairs of one side, moderately slender, av length 0·23 mm , a little more than one-third that of the fronto-clypeus, outer anterior clypeal measuring on average 0·095 mm , clypeal hair index 0·4 Posterior clypeal hairs arising a little external to the inner anterior hairs, a little shorter than the outer anterior clypeal hairs, their distal ends not reaching the base of the anterior hairs, av length 0·64 mm *Frontal hairs* normal (feathered), the distal end of the innermost pair reaching to the bases of the anterior hairs *Inner sutural hair* about

half as long as the outer anterior clypeal, simple or bifid, *outer sutural* a little shorter with 3–7 branches *Subantennal hair* stout and profusely feathered

Antenna more or less uniformly pigmented, comparatively slender, being about 9 times as long as its greatest width, a little less than half the length of the fronto-clypeus, its inner and ventro-lateral surface bearing a number of short spinous projections *Antennal hair* very short and simple, arising from the dorso-external surface about 1/3 length of antenna from base The transparent cone-shaped piece is a little longer than the short finger-shaped piece The terminal hair splits about its middle into 4–6 branches *Maxillary palp* with the cone-shaped appendage at the tip of the palp about equal to the finger-shaped appendage and a slightly bifid extreme tip *Venarium* bearing 9 teeth Of the row of four teeth on each side of the median one the anteriormost is the smallest and has a rounded end, the three anterior ones are equidistant from each other, the last one in each row being placed further back

Thorax

Submedian prothoracic hairs with the inner hair without chitinized basal tubercle, comparatively slender and bearing 7–11 branches the middle hair arising from a somewhat conspicuous basal tubercle, stout twice as long as the inner and bearing 11–14 lateral branches, the outer hair short and simple *Metathoracic palmate hair* not developed, the hair corresponding to this being like an ordinary branched hair having 3–4 branches

Pleural hairs with the arrangement of the long hairs as in group *Myzomyia* (pro- 2 simple, 1 feathered, meso- 2 simple, meta- 1 feathered 1 simple) Dorsal anterior prothoracic long, feathered, ventral long simple, dorsal posterior short with 3 lateral branches, the ventral long and simple Dorsal and ventral anterior mesothoracic long and simple, dorsal posterior extremely short, simple the ventral short, simple Dorsal anterior metathoracic long, feathered the ventral long simple, dorsal posterior minute and simple, the ventral short splitting distally into 2 The chitinous tubercles from which the pleural hairs arise are fairly large The flattened projection between the pairs of hairs is produced into a curved spine-like process on all segments, that on the mesothorax differing from the other in that instead of the outer end being prolonged into the spine it is the inner end which is so produced, in this the larva resembles species of the group *Pseudomyzomyia*

Abdomen

Hair No 1 is transferred into a poorly-developed palmate hair on segment I with 7–11 narrow lanceolate leaflets The palmate hairs are well developed on segments II–VII, though that on segment II is not so well developed as those on segments following and has only 11–13 leaflets with poorly-developed filament The other palmate hairs have 15–18 leaflets on segment III, 16–18 on segments IV–VI and 14–17 on segment VII The colour of the leaflets is uniform The filament is well differentiated with the indentations deep and somewhat restricted so that the filament is narrow at the base The average length of the blade from a mid-abdominal palmate hair is 0·055 mm and that of the filament 0·033 *Lateral hair* on segments IV–VI long, split near the base into 2 on segments IV and V and into 3 on segment VI, the corresponding hair on segment VII is very short with 3–5 branches *Post-spiracular hair* long with 4–6 branches *Saddle hair* simple *Outer submedian caudal hair* with 6–7 long stout branches the ends of which are curved to form hooks

The *anterior tergal plates* moderately large, the rounded median plate of usual size, the pair of small oval plates is absent *Spiracular apparatus* with the chitinization along the posterior border of the spiracular openings very well marked, the median plate with the anterior keel-like portion comparatively long, the plate broad anteriorly so that its latero-anterior edge touches the spiracular openings, the coloration more or less uniform *Pecten* with 4–5 long and 11–12 short spinous projections, all of which are markedly serrated along the dorso-lateral border

The accompanying chart gives the details of branching, etc, of all the hairs of the larva up to abdominal segment VII

SHOWING DETAILED CHÆTOTAXY OF THE LARVÆ OF **A. gambiæ** GILES, UP TO ABDOMINAL SEGMENT VII

Head

Hair number	2	3	4	5–7	8	9	10	11	12	13	14	15	18	20
	f	*f*	*f*	*F*	1–2	3–7	1	1–6	*LP*	1–5	2–3	2–4	Br	*F* (9)

Body

	Hair number	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Segments of thorax and abdomen	I	6–11	11–13	1	*F*	*F*	1	*F*	*F*	*F*	1	3	1	3–4	3
	II	*F*	1	1	1	3	3	3	*F*	1	1	1	1	7–9	7
	III	3–4	3	3	1	*F*	3	*F*	*F*	*F*	1	*m*	2	3	
	1	7–11	2–3	1	3–4	3–4	*F*	*F*	×	4	2–3	3	1	5–6	
	2	11–13	4–5	4–8	1	4–5	*F*	*F*	2	5–11	3–4	1	1	5–8	
	3	15–18	3–4	3–4	1	3–6	*F*	1–5	2	7–8	2–4	2–3	1	3	
	4	16–18	1	3–4	3–4	3–4	2	4–6	2	5–7	2–4	3	1	3	
	5	16–18	1–3	2–4	1	3	2	4–5	2–3	6–7	3	2–3	1	3	
	6	16–18	1	1–3	1	4–5	2–3	3–4	2–3	5–7	2–3	2–3	1	6–12	
	7	14–17	4–5	3–4	1	5–6	3–5	4–5	3–6	3–7	2	1–5	1	3	

Note.—The numbers indicate branches, *F* = feathered, *f* = frayed, *LP* = very long, pinnate, *m* = minute, × = hair absent

The other palmate hairs bear 20-21 leaflets on segments II and III 18-22 on segments IV–VI, and 16-21 on segment VII The leaflets are uniformly pigmented The filament is fairly well differentiated, the indentations at the shoulder are not very deep and the filament is fairly broad at the base in some specimens Average length of blade of leaflet 0·07 mm, that of filament 0·031 mm or a little less than half that of the blade *Lateral hairs* long, stout and profusely feathered on segments I and II that on segment III also long and stout but bearing comparatively fewer lateral branches The lateral hair is long on segments IV–VI splitting into 3-4 long branches but is very short on segment VII bearing 3-6 slender branches *Post-spiracular hair* stout bearing 5-8 long lateral branches *Saddle hair* long and simple *Outer submedian caudal* with 5 long stout branches the ends of which are curved to form hooks which are not very well developed

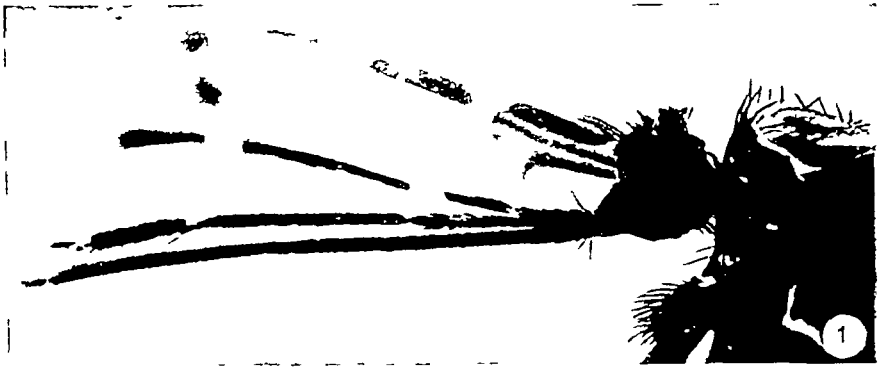
Anterior tergal plates rather small *Spiracular apparatus* with the chitinization along the posterior border of the spiracles fairly well developed The median plate is fairly broad anteriorly and touches the edge of the spiracles Pigmentation characteristic and resembling that in *A. sergenti* *Pecten* with 10-13 processes, 2 or 3 of these being a little longer than the others, all processes bear conspicuous deep serrations along the dorso-external border

The accompanying chart gives the details of branching etc of all the hairs of the larva up to abdominal segment VII

Explanation of Plate LXIII

Fig 1 Palpi, head-scales and front of thorax of *A. rhodesiensis* Theo
„ 2 Ditto of *A. dthali* Patton
„ 3 Wing of *A. theileri* Edw (Mabang specimen)
„ 4 Hind tarsus of ditto
„ 5 Wing of *A. freetownensis* Evans

The marks on the scale indicate millimetres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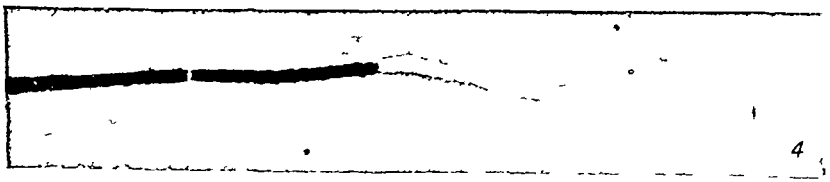
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Explanation of Plate LXIV

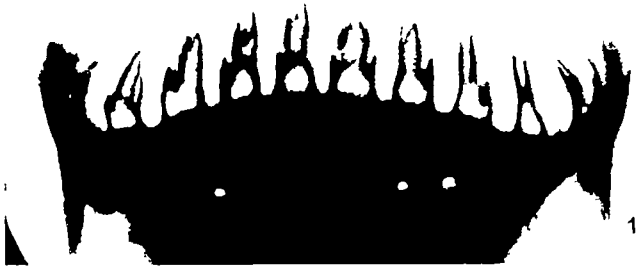
Fig 1 Pharyngeal armature of *A rhodesiensis* Theo The tops of the filaments are out of focus and indistinct

„ 2 Ditto of *A dthali* Patton

„ 3 Ditto of *A gambiæ* Giles

„ 4 Ditto of *A smithii* Theo

The marks on the scale indicate hundredths of a millimetre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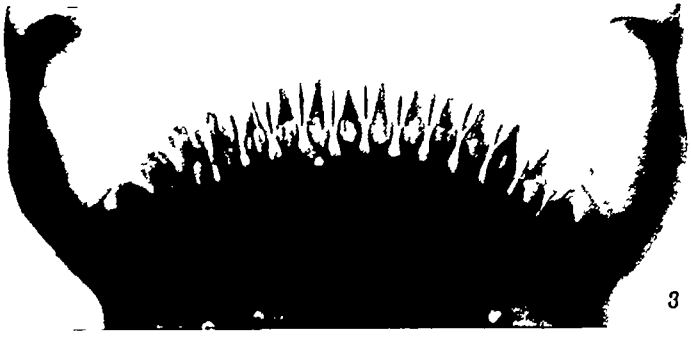
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Explanation of Plate LXV

Fig 1 Prothoracic pleural hairs of *A. theileri* Edw.
„ 2 Mesothoracic of ditto
„ 3 Metathoracic of ditto
„ 4 Mesothoracic pleural hairs of *A. gambiæ* Giles
„ 5 Prothoracic pleural hairs of *A. rhodesiensis* Theo.
„ 6 Metathoracic of ditto
„ 7 Prothoracic pleural hairs of *A. smithi* Theo.
„ 8 Metathoracic of ditto

PLATE LXV

SOME OBSERVATIONS ON BACTERIAL VARIATION IN A STRAIN OF *P SUISEPTICA* *

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THIS article is based upon my laboratory notes which I made during a serological study of 26 strains of Pasteurella group at the Lister Institute, London

It will help the interesting points in this paper to be more readily understood if I state my findings first and then the experiments which led to them

1 Two variants were isolated from a strain of *P suseptica* (National collection of type cultures No 931) one of which was agglutinable hereafter to be known as 931 A and the other slightly agglutinable or at times inagglutinable 931 I

2 The colony appearance of these variants was quite marked, the colony of 931 'A' was translucent and of 'I' fluorescent or mucoid On successive replating on ordinary agar the 'A' subculture yielded only similar colonies, whereas the 'I' subculture gave approximately 40–50 per cent 'A' type of colony and 60–50 per cent of 'I' type of colony

3 When these two variants 931 'A' and 931 'I' were grown in broth in decimal dilutions, it was observed that the 'A' variant grew in all dilutions, whereas the 'I' variant only up to 1–100,000 dilution

I METHOD OF ISOLATION OF VARIANTS

The preliminary method was, when a culture was found to be of low agglutinability, to plate from a subculture on agar and to pick 20 colonies, test their agglutinability and to select the most agglutinable variant for further use in agglutination tests This method gave an agglutinable subculture which

* A paper read before the Medical Section of the Indian Science Congress, 1930

had again become magglutinable, the subculture was again replated and 2 colonies, one translucent 'A' and the other opaque 'I,' were picked on to agar slopes and into broth and subjected to the following tests

(*a*) 'A' and 'I' subcultures were plated and 20 colonies from each were picked off on to agar slopes, subcultured and agglutination tests were carried out in a dilution of 1 in 200, with following results —

TABLE I †

931 A

1	2	3*	4	5	6*	7	8	9	10
S++	S++	—	S—	++	++	S—	++	S++	++
									Control
—	—	—	—	—	—	—	—	—	—

11	12	13	14	15	16	17	18	19	20
++	S++	+	S++	++	S++	S++	S++	++	++
									Control
—	—	—	—	—	—	—	—	—	—

* (3) and (6) selected for replating

931 I

1	2	3	4	5	6	7	8	9	10
+	+	tr	tr	S++	+	tr	tr	++	S++
									Control
—	—	—	—	—	—	—	—	—	—

11	12*	13	14	15	16	17	18	19*	20
S++	—	tr	S++	++	S++	++	tr	++	++
									Control
—	—	—	—	—	—	—	—	—	—

* (12) and (19) selected for replating

† Agglutination was recorded macroscopically as follows —

No agglutination	—
Trace of agglutination	tr
Definite agglutination	+
Agglutination with small deposit	S++
Agglutination with large deposit	++

The most agglutinable variant from 'A' was selected as well as the least agglutinable, likewise the most agglutinable and the least agglutinable variant from 'I' These were labelled $A2^{+1}$ and $A2^{-1}$ and $I2^{+1}$ and $I2^{-1}$, were subcultured and plated Ten daughter colonies of each kind were again agglutinated against the 931 serum in a dilution of 1–200 The results of such test are shown in the following table —

TABLE II

931 $A2^{+1}$

1*	2	3	4	5	6	7	8	9	10
++	++	++	++	++	++	++	++	++	++
–	–	–	–	–	–	–	–	–	–Control

$A2^{-1}$

1	2	3	4	5	1	2*	3	4	5	
++	S++	++	++	++	+	tr	++	+	r	
–	–	–	–	–	–	–	–	–	–	Control

*Colonies chosen

931 $I2^{+1}$

1	2	3	4*	5	1	2	3	4	5
tr	tr	S++	S++	+	++	++	++	++	++
–	–	–	–	–	–	–	–	–	–Control

$I2^{-1}$

1	2	3	4*	5	1	2	3	4	5	
S++	tr	+	tr	S++	S++	++	++	S++	++	
–	–	–	–	–	–	–	–	–	–	Control

*Colonies chosen

Table III given below shows the results of 931 A5++ and 931 A5-+, and 931 I5++ and 931 I5-+ in which a characteristic type of agglutination was for the first time observed associated with inagglutinability The supernatant fluid was quite clear in the agglutination tubes of all A type of colonies of both 'A' and 'I' variant The 'I' type whether derived from 'A' or 'I' variant, had a cloudy or turbid appearance

In broth, variant 'A' grows with supernatant clear and 'I' variant turbid, unlike the 'Smooth' and 'Rough' cultures described by Arkwright and others —

TABLE III

931 A5++

Supernatant fluid quite clear									
1	2	3	4	5	6	7	8	9	10
++	++	++	++	++	++			++	++
—	—	—	—	—	—	—	—	—	— Control

931 A5-+

Supernatant turbid					Supernatant quite clear					
1	2	3	4	5	1	2	3	4	5	
S++	+	++	++	++	++	++	++	++	++	
—	—	—	—	—	—	—	—	—	—	Control

'A' grows clear and 'I' turbid in broth in this type of agglutination

931 I5 +4

Supernatant turbid					Supernatant quite clear				
1	2	3	4	5	1	2	3	4	5
++	++	++	++	+	++	++	++	++	++
—	—	—	—	—	—	—	—	—	— Control

931 I5-4

Supernatant turbid					Supernatant quite clear					
1	2	3	4	5	1	2	3	4	5	
ti	ti	+	+	+	++	++	++	++	++	
—	—	—	—		—	—	—	—	—	Control

In this series 19 colonies out of 20 from A5 and 14 colonies out of 20 from I5 were agglutinable and others to a moderate degree

This method was repeated six times in accordance with the principle of selection described before and it was found that out of 20 colonies of each variant, 19 from 'A' 6 and 19 colonies from 'I' 6 were agglutinable thus demonstrating the fact that by continuous plating and picking variably agglutinating cultures may be forced to yield agglutinating cultures Table IV given below gives the results of plating a sixth time —

TABLE IV

A6+7

Supernatant quite clear									
1	2	3	4	5	6	7	8	9	10
++	++	++	++	++	++	++		++	++
−	−	−	−	−	−	−	−	−	−Control

A6 7

Supernatant turbid					Supernatant quite clear					
1	2	3	4	5	1	2	3	4	5	
++	++	++	++	++	−	++	++	++	++	
−	−	−	−	−	−	−	−	−	−	Control

I6+7

Supernatant quite clear									
1	2	3	4	5	6	7	8	9	10
++	+	++	S++	++	++	++	++	tr	++
−	−	−	−	−	−	−	−	−	−Control

I6 7

Supernatant turbid					Supernatant quite clear					
1	2	3	4	5	1	2	3	4	5	
++	S++	S++	S++	++	++	++	++	++	++	
−	−	−	−	−	−	−	−	−	−	Control

The next point tested was whether this enforced agglutinability was a permanent or temporary phase.

After four weeks in cold storage when these cultures were replated and tested, it was found that 'I' had completely reverted to its inagglutinable phase and that 'A' was slightly agglutinable. Table V gives the results of 20 colonies of each variant tested for agglutinability.

TABLE V

931 A6

1	2	3	4	5	6	7	8	9	10
+	+	S+ +	S+ +	S+	S – +	+	+	+	+
									Control
–	–	–	–	–	–	–	–	–	–
11	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
S++	S++	S++	S+–	+		+	S++	S++	S++
									Control
–	–	–	–	–	–	–	–	–	–

931 I6

1	2	3	4	5	6	7	8	9	10
tr	tr	–	tr	–	tr	+	tr	tr	tr
									Control
–	–	–	–	–	–	–	–	–	–
11	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
–	tr	–	tr	tr	tr	–	+	–	–
									Control
–	–	–	–	–	–	–	–	–	

II Characters of the colonies

The agglutinable colonies obtained in all the above tests remained translucent whereas the variably agglutinable one had become fluorescent or mucoid in appearance on the plates. When 931 A and 931 I types were plated on ordinary agar it was found that 931 A yielded 100 per cent translucent colonies and 931 I approximately 50 per cent translucent and 50 per cent mucoid colonies. When the daughter colonies from 931 'A' were replated they were

found to be almost entirely translucent colonies, and from 931 'I' plate daughter colonies the translucent colonies yielded translucent colonies, whereas the mucoid daughter colony yielded half the number mucoid and half translucent colonies These facts may be diagrammatically illustrated as follows —

Effect of Immune serum on the colony variants A6 and I6

The two variants were inoculated into broth containing small quantities of immune serum and incubated for 10 days at 37°C After this period they

DIAGRAM 1

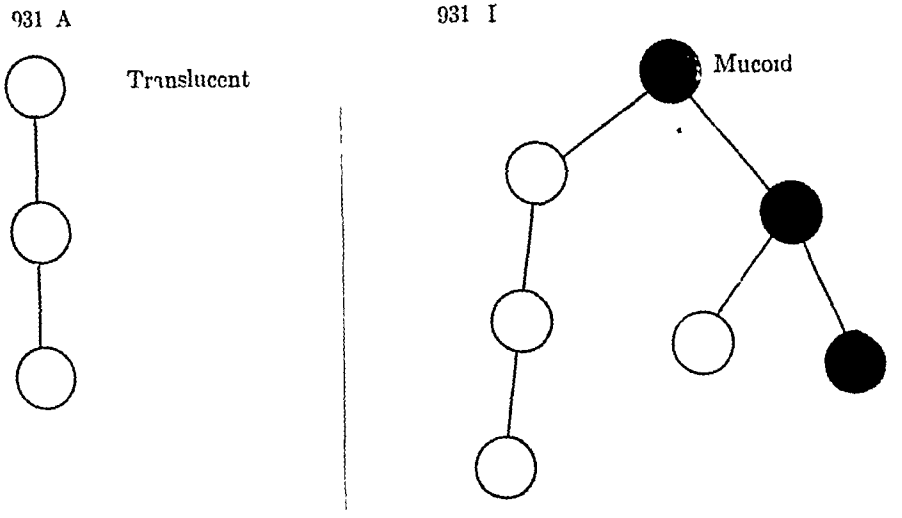
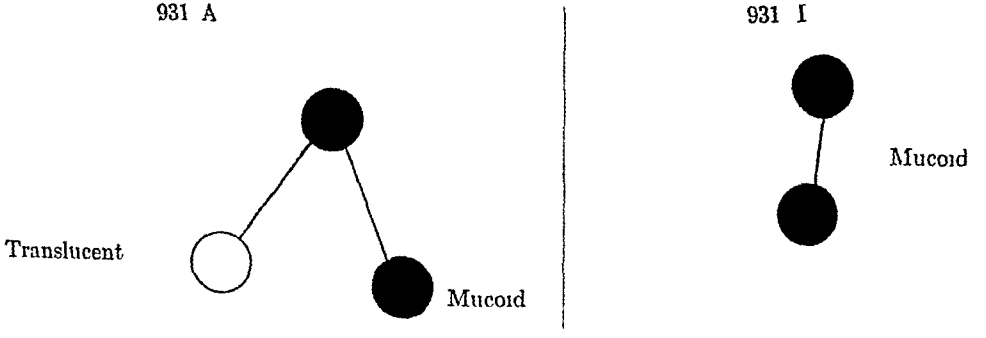


DIAGRAM 2



were plated and on examination of the colonies and counting them, it was found that the 'I' variant plate yielded all colonies of the mucoid type and the 'A' variant colonies of the mucoid and the translucent type in about equal numbers as did the 'I' variant under ordinary conditions of cultivation

Effect of augmentor on the colony variants A6 and I6 —The augmentor used in these experiments was peptonised blood containing defibrinated sheep's blood and pepsin adjusted to pH 7 0 to 7 2 with a little chloroform added to

it (Fildes, 1920), 0·5 c.c. of this augmentor was used for plating, i.e., for 15 c.c. of agar, and 1 capillary drops for one tube of broth or agar. On the augmented plates the colonies of 'A' appeared translucent as in the unaugmented plates and of 'I' some of the colonies appeared opaque and the others translucent. The variant colonies of each type 'A' and 'I' were of the same number in both unaugmented and augmented plates.

Microscopically.—The variant 'A' colony was made up of long slender bacilli, whereas the variant 'X' colony was mostly of coccoids and few short bacilli.

III. *Viability test of the 2 variants 931 A6 and 931 I6*.—The rate of growth of the 2 variants was determined as follows:—

1. *Plating method*.—After 21 hours' incubation, broth dilutions 10^{-1} to 10^{-9} were made and plated on agar (½ c.c. of the broth dilution) to determine the number of organisms per c.c. in the original broth culture.

2. *Dilution method*.—Half c.c. from each dilution from the broth culture which was plated from, was put into 1½ c.c. of broth, incubated and examined after 24, 48 and 72 hours.

Results.—1. The average count for three sets of experiments as above gave by the plating method for 'A' variant 1,200,000,000 bacilli per c.c. and for 'I' variant 110,000,000 bacilli per c.c. of original broth culture.

2. The growth in broth of 931 A6 and 931 I6 in different dilutions is given in Table VI.

TABLE VI

Growth in broth of 931 A6 and 931 I6 in different dilutions

931 A6

1/10	1/100	1/1 000	1/10 000	1/100 000	1/1m	1/10m	1/100m	1/1,000m	1/10,000m	After
++	++	++	++	++	++	++	++	++	++	24 hrs.
++	++	++	++	++	++	++	++	++	++	48 hrs.
++	++	++	++	++	++	++	++	++	++	72 hrs.

931 I6

1/10	1/100	1/1,000	1/10,000	1/100 000	1/1m	1/10m	1/100m	1/1 000m	1/10,000m	After
++	++	++	++	+	—	—	—	—	—	24 hrs.
++	++	++	++	++	+	+	+	+	+	48 hrs.
++	++	++	++	++	+	+	+	+	+	72 hrs.

By the dilution method it was found that in all dilutions variant 'A' grew but that 'I' variant did not grow in dilution above 1 in 100,000 in 24 hours There is thus seen to be a real difference existing between the 'A' and the 'I' variant in the number of bacilli present per c c as well as in the dilutions beyond which one variant will grow and the other will not

This experiment further shows that the minimum number of bacteria required for growth is much smaller in the case of A6 than of I6

Effect of augmentor on viability of 931 *A6 and* 931 *I6*.—To different broth dilutions of a 21 hours' broth culture as described in the previous experiment, 4 capillary drops of the augmentor were added, incubated at 37°C for 24 hours, 48 hours and 72 hours

The following Table VII shows that the augmentor helps the growth of 931 I6 variant in higher dilutions and it makes little or no difference in that of 931 A6 variant

TABLE VII

		1/100	1/1,000	1/10,000	1/100,000	1/1,000,000	1/10,000,000
931 I6	Without augmentor	+	+	+	+	—	—
931 I6	With augmentor	+	+	+	+	+	—
931 A6	Without augmentor	+	+	+	+	+	—
931 A6	With augmentor	+	+	+	+	+	—

SUMMARY

1 A pure culture of *P suiseptica,* 931 has been found to contain variants showing marked discontinuous variations in agglutinability This variation in agglutinability seems related to a mucoid change which the colonies undergo in subcultures

Apparently it is not related to roughness and smoothness of the colonies nor to the specific or non-specific phase of Andrews When a whole culture becomes inagglutinable, it has been found possible to pick off one or two agglutinating variants and therefore it would appear a useful point in technique to plate out the inagglutinable culture and pick about 20 colonies and test their agglutinability as an agglutinating variant may possibly be present among them

These experiments indicate individual variation among the micro-organisms constituting the parent strain and prove that daily plating and subculturing of inagglutinable cultures results in a larger number of agglutinating colonies

The necessity for subculturing affords the opportunity for growth and consequent variation to occur with the survival of a larger proportion of agglutinating variants under such conditions. Thus it would appear that any given culture consists of a large number of individual organisms which vary in one or more respects and the proportions in which the different kinds of organisms are present are variable from time to time.

Environmental conditions favour the growth of some of the types and thus lead to a gradual replacement of the agglutinating type for instance by the inagglutinable type yielding during such a change two variants showing discontinuous variations in agglutinability.

The tests on viability of the two variants 931 A6 and 931 I6 show that the augmentor helps the 'I' variant to survive in higher dilutions.

It is possible that the 'I' variant is 'oxygen sensitive' as Schutze (1929) has shown in the case of *P. pestis*.

In the light of experiments recently carried out Larson has stressed the importance of surface tension depressants in media on bacterial growth. It is likely that the surface tension requirements of the two variants may be different. This is a point worth investigating.

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EIJKMAN'S TEST APPLIED TO WATER-SUPPLIES IN THE TROPICS

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THE interpretation of the results of bacteriological examination of water-supplies in the tropics presents considerable difficulties. Lactose fermenters of the *coli* group are frequently present in very small quantities of water obtained from sources which are obviously not liable to dangerous pollution and the application of European standards as to the permissible number of these organisms would entail condemnation of many quite safe supplies.

Organisms of the *coli* group are widely distributed in natural sources in the tropics and may be of intestinal origin, human or animal, or may be present in soil under conditions which make it unlikely that they are of intestinal origin, or if of such origin, undoubtedly very remote. Such organisms must frequently be washed into water used as drinking water-supplies and the Health Officer who is presented with the results of a bacteriological examination which shows lactose fermenters to be present in quantities down to 1 c.c. or less is not justified in saying that the water shows evidence of pollution which makes it a dangerous source of supply. Most Health Officers in India have realized this and found it necessary to base their opinions on the quality of a water on the local circumstances of its collection, or to ask for special methods of bacteriological examination to be applied. In a previous paper (Taylor *et al.*, 1927) some differential methods of examination were discussed. These included the use of Clemesha's method which has been largely applied in India and consists of the identification of individual organisms and their classification according to their resistance to sunlight. This method gives some indication of the recency of pollution. The use of the methyl-red,

Voges-Proskauer, and citrate-utilization tests was found also to have a certain quantitative value as indicating the likelihood of the intestinal origin of the coliform organisms, but the exact distribution of organisms giving different types of reactions with these tests under natural conditions in soil, etc, in the tropics had not been worked out

Clemesha's method (1912) of the use of these differential tests require a considerable time to complete, and the use of special media and an extended technique Any simpler test which could be carried out rapidly and give a presumption of recent pollution from intestinal sources would be of great value

Eijkman's test is one which is used for this purpose in some countries but, so far as our survey of the literature on the subject goes, we have not been able to find any reference to the special application of this method to water-supplies in the tropics

Eijkman (1904) found that organisms capable of fermenting glucose when incubated at 46°C were characteristic of the intestinal contents of man and warm blooded animals and that organisms with this property were rarely present in nature apart from conditions under which pollution from intestinal sources was likely This was in a temperate climate He considered that incubation at 46°C for the detection of organisms of intestinal origin was a more correct procedure than incubation at 37°C or lower temperatures Eijkman did not claim that the test was one for *coli* in water although these organisms may grow at 46°C and ferment glucose, and may constitute the majority of the organisms showing this reaction

Bornand (1913) found that *B coli* after remaining in water for four-and-a-half months at low temperature developed with difficulty in Eijkman's medium at 46°C but as recency of pollution is of the greatest importance in regard to the possibility of transmission of disease by drinking water this would not appear to affect seriously the practical value of the test How far Eijkman's findings as to the distribution of organisms giving a positive result with his test apply to tropical conditions will be discussed later

Eijkman used a medium consisting of 10 per cent peptone, 10 per cent glucose and 5 per cent NaCl This was added to eight times its volume of the water to be tested in a fermentation tube or flask when large volumes were dealt with For smaller quantities such as 1 c c a 1 per cent glucose peptone water was used In each case incubation was at 46°C

His results in applying the test to water-supplies showed a marked correspondence with the sanitary circumstances of the supply, pure waters giving a negative result in quantities up to 300 c c

We have carried out a series of preliminary investigations as to the distribution and survival of lactose fermenters and of organisms giving a positive Eijkman's test under the conditions prevailing in Rangoon and the vicinity and extended the investigation to the examination of waters of different types

PRELIMINARY OBSERVATIONS

A. Fæcal samples

A series of examinations of human fæces and of the intestinal contents from the horse, cow, rabbit, guinea-pig and pigeon showed the presence of thermo-tolerant organisms producing acid and gas in 1 per cent glucose peptone water at 46°C in all samples The test was negative in the case of the intestinal contents of fish which showed lactose fermenters at 37°C

B Soil samples

Fifty samples were tested for the presence of lactose fermenters and by Eijkman's test, the details of their sources being known and the liability to contamination from human or animal sources estimated The conditions in Rangoon and the vicinity with large numbers of straying cattle, large numbers of ponies and innumerable owners dogs are such as would lead to animal contamination of soil in all unprotected sites and we have had to select special areas in order to show the degree to which sites estimated to be little liable to pollution, or well protected, will contain thermo-tolerant glucose fermenters Table I shows the results of these examinations and is divided into three sections according to the risks of pollution Of the 50 samples examined 47 or 94 per cent showed the presence of organisms fermenting lactose at 37°C and 34 or 68 per cent gave a positive Eijkman's test In the group classified as 'little liable to pollution or well protected' 22 or 58 per cent gave a positive Eijkman test, while in the groups considered 'liable to occasional pollution' or obviously 'liable to definite pollution' the tests for lactose fermenters and thermo-tolerant glucose fermenters were 100 per cent positive

In *Section A* of the table the first eight samples were carefully selected and only one gave a positive Eijkman's test This was from a railed-in enclosure protected from ordinary trespass, but a few dogs were in the area when the sample was taken Selecting six colonies after plating out on MacConkey agar from each of the lactose fermentation tubes the colonies from this series of eight samples were found to give the following reactions with the citrate utilization test, the methyl-red test and Voges-Proskauer test —

Citrate	M R	V P	No of colonies
+	—	+	43
—	+	—	3
+	+	—	2

Only 6 per cent of the colonies gave the type of reaction characteristic of intestinal *coli*

The results with regard to the samples from the Hlawga Lake catchment area which are included in the protected series are of particular interest in relation to the bacteriological results obtained from the examination of this

water-supply The catchment area extends over 10 3 square miles including 3 9 square miles of water spread It is fully fenced in and patrolled and the only persons within the area are the watchmen and a few fishermen whose sanitary conduct is regulated The greater part of the catchment area is in dense jungle difficult to penetrate and there is little inducement to trespass Cattle are excluded and there are only a few barking deer and wild fowl within the fenced area The specimens examined were obtained with difficulty from three groups of sources Those noted as 'bank' samples were obtained by approaching the shore by boat and forcing a way through thick undergrowth This area was almost unapproachable otherwise The 'outer area' samples were obtained by climbing the fencing and penetrating through the jungle in different directions No trace of human or animal trespass was noted at these points and the only paths within the catchment area are those worn by the watchmen during their patrols The 'island' samples were obtained from a small tree-covered island in the middle of the lake, of which only a few square yards were above the water level There were no animals on it, but probably birds on occasion It will be seen that the risks of human or animal pollution of these sites must be extremely small Ninety per cent of the soil samples from the catchment area showed lactose fermenters at 37°C and 70 per cent gave a positive Eijkman's test These results would suggest that organisms of these types are either naturally present in the soil or of long persistence under the climatic conditions prevailing in view of the absence of possibilities of pollution from intestinal sources

One hundred and thirty-two lactose-fermenting colonies isolated by plating from 22 positive lactose tubes of the catchment area samples gave the following reactions —

Citrate	M R	V P	No of colonies	Percentage
+	—	+	54	40 9
—	+	—	58	44
+	+	—	20	15 1

Organisms giving the reactions considered to be characteristic of *coli* of intestinal origin are obviously numerous in the soil of this protected area

In the case of the samples classified in the group considered 'liable to occasional pollution' (Table I, *Section B*) the colony reactions were —

Citrate	M R	V P	Percentage of colonies
+	—	+	69 4
—	+	—	30 6

The colonies obtained from the group classified as 'obviously liable to pollution' (Table I, *Section C*) showed —

Citrate	M R	V P	Percentage of colonies
+	—	+	72 2
—	+	—	25
+	+	—	2 8

There was thus no regular relationship between the liability to pollution of soil and the percentage of organisms isolated showing the intestinal type of reaction

It would appear that Eijkman's findings as to the distribution of thermotolerant glucose fermenters in natural sources will not apply in their entirety to tropical conditions

Survival of intestinal organisms in water and soil

These experiments were carried out to ascertain any differences there might be in regard to the relative survival of lactose fermenters at 37°C and glucose fermenters at 46°C in water and soil contaminated with fæcal matter

First water experiment—A flask of tap-water was heavily contaminated with a fresh human fæcal emulsion Samples were taken at intervals and tested for lactose fermenters and by Eijkman's test The results shown in Table II indicate a more rapid disappearance of organisms fermenting glucose at 46°C than of lactose fermenters at 37°C

Second water experiment—(Table III) The conditions were the same as in the first experiment except that the contamination with fæcal emulsion was very light and the quantities showing lactose fermenters immediately after contamination were such as would frequently be found under natural circumstances This experiment also shows the more rapid reduction of organisms giving a positive Eijkman's test The rapid divergence of the quantities in which lactose fermenters at 37°C and glucose fermenters at 46°C are present suggests that a close approximation of the quantities in which both tests are positive might be a useful indication of recency of pollution Certain examinations made of highly polluted surface waters which are described later in this paper tend to confirm this observation

Earth experiment—Dried and powdered garden earth was distributed evenly in Petri dishes and finely sprayed with a fresh fæcal emulsion A small platinum loopful of the earth was inoculated into media for tests for lactose fermenters and for Eijkman's test daily or at longer intervals The results are shown in Table IV During the month over which the tests were carried out the tests for both lactose fermenters at 37°C and glucose fermenters at 46°C were positive on all occasions Practically all colonies tested gave the reaction, Citrate — M R + V P —

TABLE I

Results of examination of soil samples classified according to their liability to pollution from human or animal sources

Section A—Sources little liable to pollution or well protected

Serial No	Source	Lactose fermenters at 37°C	Glucose fermenters at 46°C	Colonies from lactose tube				Colonies from glucose tube			
				No	Citrate	M R	V P	No	Citrate	M R	V P
1	Enclosed lawn, Turf Club No 1	+	—	6	+	—	+				
2	Enclosed lawn, Turf Club No 2	+	—	6	+	—	+				
3	Enclosure, high level tank, Kokine	+	+	6	+	—	+				
4	Lawn, Mingaladon bungalow	+	—	6	+	—	+				
5	Main lawn, Director's compound	+	—	6	+	—	+				
6	Small lawn, Director's compound	+	—	5	+	—	+				
				1	—	+	+				
7	Croton bed, Director's compound	+	—	4	+	—	+				
				1	+	+	—				
				1	—	+	—				
8	Lawn, Patients' quarters	+	—	1	+	—	+				
				1	+	+	—				
				1	—	+	—				
	Catchment area, Hlagwa Lake—										
9	No 1 'Bank' sample	+	+	3	+	—	+	6	—	+	—
				3	—	+	—				
10	No 2 „ „	+	+	4	—	+	—				
				2	+	—	+				
11	No 3 „ „	+	—	3	+	—	+				
				3	—	+	—				

12	No 4 „ „	+	−	1 1 1	+ + −	− + +	+ − −					
13	No 5 „ „	+	−	1 2	+ −	− +	+ −					
14	No 6 „ „	+	+	1 2	− +	+ −	− +	6	−	+	−	
15	No 7 „ „	+	+	3 3	+ −	− +	+ −					
16	No 8 „ „	+	+	5 1	+ +	− +	+ −					
17	No 9 „ „	+	+	6	−	+	−	6	−	+	−	
18	No 10 „ „	+	+	5 1	− +	+ −	− +					
19	No 11 'Outer area' sample	+	+	4 2	− +	+ +	− −	6	+	−	+	
20	No 12 „ „ „	+	+	4 2	− +	+ −	− +	3 2 1	+ + −	− + +	+ − −	
21	No 13 „ „	+	+	5 1	− +	+ −	− +	6	−	+	−	
22	No 14 „ „ „	+	+	3 2 1	− + +	+ − +	− + −	6	−	+	−	
23	No 15 „ „ „	+	+	3 2 1	+ + −	+ − +	− + −	6	−	+	−	
24	No 16 „ „ „	−	−									

Note—The results of fermentation tests shown in this and subsequent tables were recorded after 48 hours' incubation at the given temperature

TABLE I—*contd*

Section A—*concld*

Serial No	Source	Lactose fermenters at 37°C	Glucose fermenters at 46°C	Colonies from lactose tube				Colonies from glucose tube			
				No	Citrate	M R	V P	No	Citrate	M R	V P
	Catchment area, Hlawga Lake—										
25	No 17 Outer area sample	—	—								
26	No 18 „ „ „	—	—								
27	No 19 „ „ „	+	+	1 2	— +	+ —	— +	6	—	+	—
28	No 20 „ „ „	+	—	5 1	+ —	— +	+ —				
29	No 21 „ „ „	+	—	3 3	+ +	— +	+ —				
30	No 22 „ „ „	+	+	5 1	+ +	+ —	— +	6	+	—	+
31	No 23 „ „ „	+	+	3 3	+ —	— +	+ —	6	+	—	+
32	No 24 „ „ „	+	+	4 2	+ —	— +	+ —	3 2 1	+ — +	+ + —	— — +
33	No 25 „ „	+	+	4 2	+ +	+ —	— +	5 1	— +	+ —	— +

34	No 26 'Island sample' (Hlawga Lake)	+	+	Not tested							
35	No 27 „ „	+	+	„ „							
36	No 28 „ „	+	+	„ „							
37	No 29 „ „	+	+	„ „							
38	No 30 „ „	+	—	„ „							

Section B—Sources liable to occasional pollution

Serial No	Source	Lactose fermenters at 37°C	Glucose fermenters at 46°C	Colonies from lactose tube				Colonies from glucose tube			
				No	Citrate	M R	V P	No	Citrate	M R	V P
39	Institute compound	+	+	6	+	—	+	6	—	+	—
40	Church Road garden No 1	+	+	5 1	+ —	— +	+ —	6	—	+	—
41	Church Road garden No 2	+	+	5 1	— +	+ —	— +	6	—	+	—
42	Chemical Examiner's compound	+	+	4 2	+ —	— +	+ —	4 2	— +	+ —	— +
43	Cross Roads garden, Godwin Road	+	+	6	+	—	+	6	—	+	—
44	Artillery Headquarters' compound	+	+	3 3	— +	+ —	— +	6	+	—	+

TABLE I—*concld*

Section C—Sources obviously liable to pollution

Serial No	Source	Lactose fermenters at 37°C	Glucose fermenters at 46°C	Colonies from lactose tube				Colonies from glucose tube			
				No	Citrate	M R	V P	No	Citrate	M R	V P
45	Dry roadside ditch, Stewart Road	+	+	5	—	+	—				
				1	+	—	+				
46	Dry roadside ditch, Church Road	+	+	6	+	—	+	6	—	+	—
47	Bedside bathing platform, Church Road	+	+	4	+	—	+	6	—	+	—
				2	—	+	—				
48	Bedside servants' quarters, Church Road	+	+	3	+	—	+	6	—	+	—
				2	—	+	—				
				1	+	+	—				
49	Grazing ground, West Bazaar Road	+	+	6	+	—	+				
50	Exercising track, Race Course	+	+	6	+	—	+	6	—	+	—

TABLE II

Showing survival of intestinal organisms in water

(Sample of tap-water heavily contaminated with fæcal emulsion)

Period after contamination	Lactose fermenters at 37°C	Glucose fermenters at 46°C	Colonies from lactose tube			
			No	Citrate	M R	V P
Before contamination	Present in 10 c.c.	Present in 50 c.c.	6	+	+	−
Immediately after contamination	„ „ 0·001 c.c.	„ „ 0·001 c.c.	6	−	+	−
1 day after contamination	„ „ 0·001 c.c.	„ „ 0·005 c.c.	6	−	+	−
2 days after contamination	„ „ 0·001 c.c.	„ „ 0·005 c.c.	6	−	+	−
3 „ „ „	„ „ 0·005 c.c.	„ „ 0·05 c.c.	6	−	+	−
4 „ „ „	„ „ 0·005 c.c.	„ „ 0·05 c.c.	6	−	+	−
5 „ „ „	„ „ 0·05 c.c.	„ „ 0·05 c.c.	6	−	+	−
6 „ „ „	„ „ 0·01 c.c.	„ „ 0·05 c.c.	6	−	+	−
7 „ „ „	„ „ 0·01 c.c.	„ „ 0·05 c.c.	6	−	+	−
8 „ „ „	„ „ 0·01 c.c.	„ „ 0·5 c.c.	5	−	+	−
			1	+	−	+
10 „ „ „	„ „ 0·1 c.c.	„ „ 0·5 c.c.	6	−	+	−
13 „ „ „	„ „ 0·1 c.c.	„ „ 5 c.c.	6	−	+	−
15 „ „ „	„ „ 0·1 c.c.	„ „ 10 c.c.	6	−	+	−
17 „ „ „	„ „ 0·5 c.c.	„ „ 10 c.c.	6	−	+	−
20 „ „ „	„ „ 0·5 c.c.	„ „ 10 c.c.	6	−	+	−
22 „ „ „	„ „ 0·5 c.c.	„ „ 25 c.c.	6	−	+	−
24 „ „ „	„ „ 1 c.c.	„ „ 25 c.c.	6	−	+	−
27 „ „ „	„ „ 1 c.c.	Absent from 50 c.c.	5	−	+	−
			1	+	+	−
30 „ „ „	„ „ 5 c.c.	„ 100 c.c.	5	−	+	−
			1	+	+	−
33 „ „ „	„ „ 10 c.c.	„ „ 100 c.c.	6	−	+	−
36 „ „ „	„ „ 10 c.c.	„ „ 100 c.c.	6	−	+	−

TABLE III

Showing the survival of intestinal organisms in water (Tap-water lightly contaminated with fæcal emulsion)

Period after contamination	Lactose fermenters at 37°C	Glucose fermenters at 46°C	Colonies from lactose tube			
			No	Citrate	M R	V P
Before contamination	Present in 50 c c	Present in 50 c c	6	+	−	+
Immediately after contamination	„ „ 0·1 c c	„ „ 0·1 c c	1	−	+	−
			2	+	−	+
1 day after contamination	„ „ 0·1 c c	„ „ 0·1 c c	1	+	−	+
			2	−	+	−
2 days after contamination	„ „ 0·05 c c	„ „ 0·01 c c	1	−	+	−
			2	+	−	+
4 „ „ „	„ „ 0·1 c c	„ „ 0·5 c c	6	−	+	−
5 „ „ „	„ „ 0·1 c c	„ „ 10 c c	3	−	+	−
			3	+	−	+
7 „ „ „ ..	„ „ 0·1 c c	„ „ 10 c c	1	+	−	+
			2	−	+	−
9 „ „ „ .	„ „ 0·5 c c	„ „ 10 c c	3	+	−	+
			2	−	+	−
			1	+	+	−
11 „ „ „	„ „ 0·5 c c	„ „ 25 c c	5	−	+	−
			1	+	−	+
14 „ „ „ ..	„ „ 5 c c	„ „ 10 c c	6	−	+	−
16 „ „ „ ..	Absent from 50 c c	„ „ 50 c c	No growth			
18 „ „ „ ..	Present in 100 c c	„ „ 100 c c	6	+	−	+
21 „ „ „ ..	„ 100 c c	Absent from 100 c c	6	+	−	+

TABLE IV

Showing survival of intestinal organisms in soil
(Dried garden earth in Petri dishes contaminated with fæcal emulsion)

Period after contamination	Lactose fermenters at 37°C	Glucose fermenters at 16°C	Colonies from lactose tube			
			No	Citrate	M R	V P
Before contamination	+	+	6	+	−	+
Immediately after contamination	+	+	4	−	+	−
			1	+	+	−
			1	+	−	+
1 day after contamination	+	+	6	−	+	−
2 days after contamination	+	+	6	−	+	−
3 „ „ „	+	+	6	−	+	−
5 „ „ „	+	+	6	−	+	−
6 „ „ „	+	+	6	−	+	−
7 „ „ „	+	+	6	−	+	−
9 „ „ „	+	+	6	−	+	−
13 „ „ „	+	+	6	−	+	−
16 „ „ „	+	+	6	−	+	−
18 „ „ „	+	+	6	−	+	−
21 „ „ „	+	+	6	−	+	−
24 „ „ „	+	+	6	−	+	−
27 „ „ „	+	+	6	−	+	−
30 „ „ „	+	+	6	−	+	−
33 „ „ „	+	+	6	−	+	−

The tests were not done quantitatively and it was not possible to say whether there was any relative reduction in the organisms giving a positive Eijkman's test The temperature of the laboratory at the time of the experiment showed a mean of about 80°F and a diurnal variation of about 10°F The observations were not carried further but show that organisms fermenting glucose at 46°C have a considerable period of survival under the climatic conditions present

EXAMINATION OF WATER SAMPLES

Examinations were first made of water from shallow wells, tanks and other surface collections of water considered liable to definite pollution For comparison a series of examinations of tube-well waters were made which could be considered to be safe from any chance of pollution or in some degree defective in this respect The series of samples which was considered to be of the greatest importance for our purposes was that from the Hlawga Lake reservoir, a type of water-supply in regard to which the interpretation of the results of bacteriological examinations has always presented considerable difficulties under tropical conditions

A Shallow well samples

A series of samples from shallow wells were examined, quantities of 1 c c and upwards being put up in the case of the first samples and later samples being tested in quantities down to 0 001 c c

The following are the details of the wells and the results given by the bacteriological examinations —

No 1 *Hlegu Bazaar well*—Brick and cement built with coping and platform Water eight feet from surface Surrounded by shops and houses Decomposing rubbish on ground

Lactose fermenters at 37°C	present in 1 c c or less
Glucose „ „ 46°C	„ „ 10 c c
Colonies from lactose tube	6, Citrate — M R + V P —

No 2 *Hlegu Hindu temple well*—Brick lined and pointed Uncemented coping and platform Water four feet from surface

Lactose fermenters at 37°C	present in 1 c c or less
Glucose „ „ 46°C	„ „ 10 c c
Colonies from lactose tube	4, Citrate + M R — V P +
	2, Citrate — M R + V P —

No 3 *U San Kyaw's well, Hlegu*—Brick lined, cemented coping and platform Water four and a half feet from surface Drain with stagnant water ten feet off and dwelling houses within fifteen feet

Lactose fermenters at 37°C	present in 1 c c or less
Glucose „ „ 46°C	„ „ 10 c c

No 4 Dak Bungalow well, Hlegu—Brick and cement lined with coping and platform Surroundings covered with weeds and vegetation Roadside drain close by Said to be leakage into well Water one foot from surface

Lactose fermenters at 37°C	present in 1 c c or less
Glucose „ „ 46°C	„ „ 10 c c

No 5 Minmigaon well, Rangoon—Brick lined but uncemented Cement coping No platform Water 30 feet from surface Close to road and houses Bucket latrine within 20 yards

Lactose fermenters at 37°C	present in 1 c c or less
Glucose „ „ 46°C	„ „ 10 c c
Colonies from lactose tube	4, Citrate + M R — V P +
	2, Citrate — M R + V P —
Colonies from glucose tube	6, Citrate — M R + V P —

No 6 U Ba Han's garden well—Old well with perished brick lining Broken down coping No platform Water 50 feet from surface Surrounded by decaying vegetation and weeds, but some distance from dwellings Used for garden watering

Lactose fermenters at 37°C	present in 1 c c or less
Glucose „ „ 46°C	„ „ 50 c c
Colonies from lactose tube	5, Citrate + M R — V P +
	1, Citrate — M R + V P —
Colonies from glucose tube	5, Citrate — M R + V P —
	1, Citrate + M R — V P +

No 7 Experimental well boring in compound of Hygiene Institute—A series of short tube-wells had been placed around a bore-hole latrine to test the degree of pollution occurring

Lactose fermenters at 37°C	present in 0 05 c c
Glucose „ „ 46°C	„ „ 0 5 c c
Colonies from lactose tube	5, Citrate — M R + V P —
	1, Citrate + M R + V P —
Colonies from glucose tube	6, Citrate — M R + V P —

No 8 Phoongyi Chaung well, Boundary Road—Brick and cement lined with coping and platform Water 15 feet from surface Close to shops and in dirty surroundings

Lactose fermenters at 37°C	present in 1 c c
Glucose „ „ 46°C	„ „ 0 1 c c
Colonies from lactose tube	5, Citrate + M R — V P +
	1, Citrate — M R + V P —
Colonies from glucose tube	5, Citrate + M R — V P +
	1, Citrate — M R + V P —

No 9 *Well in compound No* 202 *Boundary Road*—Well built with cement lining, coping and platform Water 30 feet from surface Surroundings fairly clean but servants' quarters within 25 feet

Lactose fermenters at 37°C	present in 1 c c
Glucose „ 46°C	„ „ 1 c c
Colonies from lactose tube	5, Citrate — M R + V P — 1 Citrate + M R — V P +
Colonies from glucose tube	5, Citrate + M R — V P + 1, Citrate — M R + V P —

No 10 *Well at* 11 *mile Prome Road*—Old brick lining unecmented Broken coping No platform Situated on roadside within a few feet of houses Water four feet from surface

Lactose fermenters at 37°C	present in 0 05 c c
Glucose „ „ 46°C	0 5 c c
Colonies from lactose tube	4 Citrate + M R — V P + 2 Citrate — M R + V P —
Colonies from glucose tube	4 Citrate + M R — V P + 2, Citrate — M R + V P —

All these samples were from shallow wells and in the case of the majority of them the surroundings were such as to indicate the extreme liability of pollution Lactose fermenters were present in 1 c c or less in all samples and a proportion of the lactose fermenters isolated from them showed the intestinal type of reaction, viz, Citrate — M R + V P —

Organisms fermenting glucose at 46°C were present in 10 c c or less in all samples with the exception of No 6 in which they were present in 50 c c only This was a well which although in untidy surroundings was of considerable depth and did not appear to be likely to receive gross immediate contamination from human or animal sources

Sample No 7 was obtained from an experimental source in which conditions leading to latrine contamination had been specially devised and showed thermo-tolerant glucose fermenters in 0 5 c c, the colonies from the glucose tube being all of the intestinal type While on the whole the results with the citrate utilization and methyl-red tests correspond with the local circumstances of the samples the results were by no means regular and the proportion of colonies showing the intestinal type of reaction did not always correspond with the relative degree of pollution to which the source of supply was liable

B Tanks and other unprotected surface waters

A few samples were obtained from tanks consisting of surface collections of water retained by embankments, of which some were used as source of drinking water-supply Other casual water and roadside samples are included in this series

No 1 *Hlegu East Village tank*—Unprotected by fencing High embankment around No platform for drawing water Individuals walk into tank to draw water Size 140 feet by 60 feet

Lactose fermenters at 37°C	present in 1 c c or less
Glucose „ „ 46°C	„ „ 10 c c

No 2 *Hlegu Chettiar's temple tank*—Protected from animal trespass by bamboo fence Full of weeds and vegetation No platform for drawing water Water drawn by wading into tank

Lactose fermenters at 37°C	present in 1 c c or less
Glucose „ „ 46°C	„ „ 10 c c

No 3 *Theatre Road tank*—Surrounded by partial embankment Liable to receive road and surface drainage Not used for drinking

Lactose fermenters at 37°C	present in 0 5 c c
Glucose „ , 46°C	„ „ 5 c c
Colonies from lactose tube	6, Citrate + M R — V P +
Colonies from glucose tube	6, Citrate — M R + V P —

No 4 *Cantonment Garden Lake*—Not embanked Receives road and surface drainage

Lactose fermenters at 37°C	present in 0 1 c c
Glucose „ , 46°C	„ „ 10 c c
Colonies from lactose tube	5, Citrate + M R — V P +
	1, Citrate — M R + V P —

No 5 *Pond below Pagoda in centre of cattle grazing ground*—

Lactose fermenters at 37°C	present in 0 5 c c
Glucose „ „ 46°C	„ „ 1 c c
Colonies from lactose tube	3, Citrate — M R + V P —
	6, Citrate + M R — V P +
Colonies from glucose tube	6, Citrate — M R + V P —

No 6 *Roadside drain, Hanthawaddy Road*—In front of shops and dwelling houses Receives rubbish and other polluting matter from shops

Lactose fermenters at 37°C	present in 0 001 c c
Glucose „ „ 46°C	„ „ 0 001 c c
Colonies from lactose tube	6, Citrate — M R + V P —

No 7 *Roadside drain, Boundary Road*—Receives surface drainage

Lactose fermenters at 37°C	present in 0 01 c c
Glucose „ „ 46°C	„ „ 0 01 c c
Colonies from lactose tube	5, Citrate — M R + V P —
	1, Citrate + M R — V P +.
Colonies from glucose tube	6, Citrate — M R + V P —

Lactose fermenters were present in 1 c c or less in all samples and in smaller quantities when tested Glucose fermenters were present in 10 c c in all samples and in quantities down to 0 001 c c in some cases when the source was obviously liable to heavy pollution The incidence of thermo-tolerant glucose fermenters was in a fair degree proportionate to the relative chances of recent pollution None of the sources could be considered safe for drinking water-supply in view of their local circumstances

C Tube-well samples

A series of samples from tube-wells in and around Rangoon were examined, the samples being collected direct from the tanks into which the water was pumped Quantities up to 50 c c were as a rule examined but larger quantities were tested in some instances

1 *Windermere Park Tube-well*—This is a good well in satisfactory surroundings supplying the houses of a Government estate It was constructed in 1922 and has a depth of 85 feet and a delivery of 6 000 gallons per hour

1st sample

Lactose fermenters at 37°C	absent from	100 c c
Glucose „ „ 46°C	„	100 c c

2nd sample

Lactose fermenters at 37°C	absent from	100 c c
Glucose „ „ 46°C	„ „	100 c c

3rd sample

Lactose fermenters at 37°C	present in	50 c c
Glucose „ „ 46°C	absent from	350 c c

The water is of a high standard of bacteriological purity The presence of lactose fermenters in 50 c c in one sample cannot be considered to be of any significance in view of the results of previous examinations and the absence of thermo-tolerant glucose fermenters from 350 c c

2 *Kemmendine Tube-well*—A well of high capacity operated by a private firm pumping into the mains supplying one area of Rangoon The well is modern and of good construction

Lactose fermenters at 37°C	absent from	50 c c
Glucose „ „ 46°C	„ „	50 c c

3 *Leper Asylum Tube-well*—A small well constructed in 1922 Depth 118 feet and capacity 1,930 gallons per hour

Lactose fermenters at 37°C	absent from	50 c c
Glucose „ „ 46°C	„ „	50 c c

4 *Rangoon Jail Tube-well*—This well was constructed in 1910 and has a depth of 165 feet and a yield of 3,300 gallons per hour It is reported to have no defects but the pumping level is 27½ feet and the rest water-level 11 feet This well has not always given as good results as should be expected

Lactose fermenters at 37°C	present in 10 c c
Glucose „ „ 46°C	absent from 50 c c
Colonies from lactose tube	3, Citrate + M R — V P +
	2, Citrate — M R + V P —
	1, Citrate + M R + V P —

The presence of lactose fermenters in 10 c c of this tube-well water might be considered suspicious and the type of colony present might suggest the occurrence of some degree of pollution

The negative Eijkman test in 50 c c however would indicate that at the time of taking the sample no dangerous pollution was occurring

5 *Kyundau Tube-well*—This well supplies one area of Rangoon city, pumping into the mains It was constructed in 1927 and has a depth of 115 feet and a yield of 3,500 gallons per hour

Lactose fermenters at 37°C	present in 10 c c
Glucose „ „ 46°C	absent from 50 c c
Colonies from lactose tube	6, Citrate + M R — V P +

The presence of lactose fermenters in 10 c c might be considered unsatisfactorily in a tube-well but the results of Eijkman's test conform with the local circumstances of the well which are good The well would not appear to be liable to pollution and the Chief Engineer states that it has no defects

6 *Ahlone Tube-well*—This is an old well made in 1904 to a depth of 230 feet giving an average yield of 8,000 gallons per hour The water is pumped into the city mains It is situated in unsatisfactory surroundings in the vicinity of a night-soil depot

There is no record of the well having been cleaned since its construction and it has not been examined for defects It is suggested that the piping may have become porous from age

Lactose fermenters at 37°C	present in **5 c c**
Glucose „ „ 46°C	present in 25 c c
Colonies from lactose tube	6, Citrate — M R + V P —
Colonies from glucose tube	6, Citrate — M R + V P —

The results of the Eijkman test would suggest a strong suspicion of defect and contamination of this well and the colony characteristics would strengthen such suspicion

7 *University Estate Tube-wells*—Two wells providing the main portion of the supply were examined These were situated close to each other in a site free from habitation which has recently been cleared from jungle and in which there is no source of pollution The wells were constructed in 1927 and are

of 12 inch diameter Well No 1 has a depth of 123 feet and a yield of 8,000 gallons per hour Well No 2 has a depth of 144 feet and a yield of 9,500 gallons per hour These wells from their construction and surroundings can be considered to be free from risks of contamination

Well No 1

Lactose fermenters at 37°C	present in 10 c c
Glucose „ „ 46°C	„ „ 100 c c
Colonies from lactose tube	3, Citrate — M R + V P —
	3, Citrate + M R + V P —
Colonies from glucose tube	6, Citrate + M R — V P +

Well No 2

Lactose fermenters at 37°C	present in 10 c c
Glucose „ „ 46°C	„ „ 100 c c
Colonies from lactose tube	6, Citrate — M R + V P —

As in previous samples the presence of lactose fermenters in 10 c c of a tube-well water would be suspicious and some of the colonies were of the type giving the reactions usually shown by intestinal organisms The Eijkman's test positive in 100 c c but not in lesser quantities would remove suspicion of contamination which is in line with the findings as to the local circumstances of these wells

In this series of tube-well water samples all except one well in which there is suspicion of defect gave a negative Eijkman's test in 50 c c In two samples from wells which are above suspicion the test was positive in 100 c c but not in lesser quantities Negative results in 100 c c and up to 350 c c were shown in some samples The tests for lactose fermenters gave varying results and these organisms were present in 10 c c in samples from wells free from suspicion of pollution

Lactose fermenters were present in 5 c c in the Ahlone tube-well which also showed all colonies tested to give the reaction, Citrate — M R + V P — This is an old and probably defective well badly situated

The presence of lactose fermenters in 5 c c of this well water compared with their presence in 10 c c of the University Estate wells would not suggest much difference between these but the Eijkman's test being positive in 25 c c in the former and negative in lesser quantity than 100 c c in the two latter wells gives an indication of possible pollution which is in accordance with the relative risks of these sources of supply The colony characteristics do not help in differentiating the qualities of these waters

The Eijkman's test would appear to be a better guide to the occurrence of pollution in these wells than the other tests applied The wide distribution of organisms fermenting glucose at 46°C in the conditions prevailing in Rangoon and the vicinity appear to necessitate a lower standard for these organisms

than would be acceptable in Europe and we suggest that a suitable standard for tube-well waters in the tropics is 'Eijkman's test to be negative in quantities less than 100 c c'

D Hlawga Lake reservoir samples

Hlawga lake forms the main source of the piped water-supply of Rangoon The lake is situated about 17 miles from the city and has been artificially constructed by the formation of an embankment between low hills It drains a catchment area of 10 3 square miles and has a water spread of 3 9 square miles The conditions in the catchment have already been described in connection with the examination of soil samples and it can be considered to be very well protected and little liable to trespass or pollution As has been noted, in spite of the protected nature of the catchment area, 90 per cent of soil samples from it showed the presence of lactose fermenters and 70 per cent gave a positive Eijkman's test It is obvious that, especially in the season of heavy monsoon rains, these organisms must be washed into the lake and their presence would not necessarily indicate pollution of recent or dangerous nature

An examination of water samples from this source might be expected to show the normal limits within which certain types of organisms may be present, apart from pollution, in a protected upland surface water in the tropics Sixteen samples were examined over a period of about two months and the results are shown in Table V Larger quantities than 50 c c were not tested

The results with the tests for lactose fermenters at 37°C were very irregular These organisms were present in quantities varying from 0 5 c c to 25 c c as under —

Quantity in which present	No of samples
25 c c	1
10 c c	6
5 c c	4
1 c c	3
0 5 c c	2

The following are the percentages of the colonies examined which were obtained by plating out on MacConkey agar from the positive lactose tubes giving different types of reactions with the citrate utilization, methyl-red, and Voges-Proskauer tests —

Citrate	M R	V P	Percentage of colonies
—	+	—	49
+	—	+	32 3
+	+	—	18 7

TABLE V

Hlawga Lake samples

Serial No	Date	Lactose fermenters at 37°C	Glucose fermenters at 46°C	Colonies from lactose tube				Colonies from glucose tube				
				No	Citrate	M R	V P	No	Citrate	M R	V P	
1	24-7-30	Present in 10 c c	Absent from 50 c c	6	+	—	+					
2	4-8-30	„ , 0·5 c c	„ „ 50 c c	5	+	—	+					
				1	+	+	—					
3	6-8-30	„ „ 5 c c	Present in 50 c c	5	+	—	+	6	+	—	+	
				1	—	+	—					
4	7-8-30	„ , 5 c c	„ 50 c c	5	—	+	—	Not tested				
				1	+	+	—					
5	10-8-30	„ „ 1 c c	„ , 50 c c	6	+	—	+	6	+	—	+	
6	11-8-30	„ , 5 c c	Absent from 50 c c	1	+	+	—					
				2	—	+	—					
7	18-8-30	„ „ 1 c c	„ , 50 c c	1	—	+	—					
				2	+	+	—					
8	19-8-30	„ „ 1 c c	„ , 50 c c	6	+	—	+					
9	21-8-30	„ „ 5 c c	Present in 50 c c	5	+	+	—	Not tested				
				1	+	—	+					
10	25-8-30	„ „ 10 c c	„ „ 50 c c	6	—	+	—	6	—	+	—	
11	26-8-30	„ „ 25 c c	Absent from 50 c c	5	—	+	—					
				1	+	—	+					
12	28-8-30	„ „ 10 c c	Present in 50 c c	4	—	+	—	Not tested				
				1	+	+	—					
				1	+	—	+					
13	1-9-30	„ „ 10 c c	Absent from 50 c c	4	—	+	—					
				2	+	+	—					
14	2-9-30	„ „ 10 c c	„ „ 50 c c	4	—	+	—					
				2	+	+	—					
15	8-9-30	„ „ 0·5 c c	Present in 50 c c	6	—	+	—	6	—	+	—	
16	12-9-30	„ „ 10 c c	„ „ 50 c c	6	—	+	—	6	—	+	—	

The high percentages of organisms giving reactions which are similar to those shown by organisms of intestinal origin might suggest the occurrence of pollution from human or animal sources but from the description which has been given of the catchment area it will be apparent that such pollution is extremely unlikely and could certainly not be responsible for the very marked variation in the results given both by the tests for lactose fermenters and for the types of organisms present If Table V be examined in detail it will be seen that the quantities in which lactose fermenters are present have no correlation with the presence of organisms giving the reactions which are considered to be of the intestinal type For example, sample No 2 shows lactose fermenters present in 0 5 c c but no organisms giving the reaction, Citrate — M R + V P — while in the case of sample No 10 in which lactose fermenters are only present in 10 c c all colonies tested were of that type A comparison of other samples would show similar differences The results with the Eijkman's test are, in contrast, very regular Glucose fermenters at 46°C are either absent from 50 c c or present in this quantity but not in lesser amounts except in the case of sample No 5 which was collected by wading into the lake and in the process a certain amount of mud was stirred up The other samples were collected by boat at the intake

In the samples in which the Eijkman's test was positive in 50 c c the colony reactions varied The regularity of the results with the Eijkman's test is in conformity with our observations on the local conditions which show that this supply is not liable to dangerous pollution or to marked variations in risks in this regard Any serious pollution would be expected to result in considerable variations in the quantities in which the Eijkman's test would be positive In view of the results of examination of soil samples from the catchment area it would be expected that organisms fermenting glucose at 46°C would be present in the water in some proportion and the results of these tests would suggest that a suitable standard of purity for a protected upland surface water of this nature in the tropics would be 'a negative Eijkman's test in quantities less than 50 c c'

Discussion

The examination of soil samples in Rangoon and the vicinity has shown the almost universal distribution of organisms fermenting lactose at 37°C Organisms fermenting glucose at 46°C were also widely distributed but were found in a somewhat smaller proportion of samples Both types of organisms were found under conditions in which there was no likelihood of recent contamination from intestinal sources and even where there were special measures of protection against fæcal pollution Of the lactose fermenters isolated from soil, a considerable proportion gave the reactions with the citrate utilization, methyl-red and Voges-Proskauer tests usually considered to be typical of *coli* of intestinal origin

Experimentally both lactose fermenters and organisms fermenting glucose at 46°C were found to have a prolonged period of survival in soil

Under the circumstances it is to be expected that both lactose fermenters and organisms giving a positive Eijkman's test will be found in some proportion in waters of surface origin in the area of Lower Burma in which the tests were carried out even if collected from catchment areas not liable to pollution The series of examinations carried out on the water of the Hlawga Lake reservoir which is a well protected source of water-supply of surface origin were undertaken in order to ascertain what were the ordinary limits of lactose fermenters and organisms giving Eijkman's test and to what extent these tests corresponded with sanitary circumstances of the supply The volume of the lake and the very minor possibilities of casual pollution make it unlikely that contamination from intestinal sources could produce any marked variations in the content of *coliform* organisms from such sources but at the time of the observations, during the heavy south-west monsoon ground washings from the protected catchment area the soil of which shows a wide distribution of lactose fermenters and organisms giving a positive Eijkman's test might result in an increase in bacterial content It was found that lactose fermenters were present in quantities varying from 0 5 c c to 25 c c in samples taken at frequent intervals over a period of two months A varying degree of marked pollution from intestinal sources to give such changes could not have occurred The types of organisms present did not have the correspondence with the quantities in which lactose fermenters were present which would be expected *if contamination was of intestinal origin For example, one sample* showed lactose fermenters in 0 5 c c and all colonies tested gave the reactions, Citrate + M R — V P + while from another showing lactose fermenters in 10 c c all colonies were Citrate — M R + V P — The results with Eijkman's test were on the other hand very regular, this test being either negative in 50 c c or positive in that quantity but not in lesser amounts It would be very difficult to give an opinion of the sanitary quality of this water on the results of the tests for lactose fermenters in view of the wide variations almost from day-to-day, and the types of organisms isolated are also of little assistance The regularity of the results with the Eijkman's test are in conformity with our estimate of the risks of pollution and this test would appear to furnish a true indication as to the quality of the water We have shown that organisms giving a positive Eijkman's test were present in a considerable proportion of samples from well protected parts of the catchment area and we must accordingly expect to find some organisms of this type in the water A European standard for Eijkman's test is properly not applicable and we suggest that a suitable standard for surface waters of this type in the tropics would be 'Eijkman's test to be negative in quantities less than 50 c c'

For comparison we have tested a series of shallow well waters and surface waters liable to pollution In all cases Eijkman's test was positive in 10 c c or less and where gross pollution was present the test was in some cases positive in as small a quantity as 0 001 c c When positive in small quantities the tests for lactose fermenters were positive in similar amounts, a point which

had already been mentioned in regard to recency of pollution In some cases the tests for lactose fermenters in shallow wells liable to pollution were positive in 1 c c only which is no larger quantity than was sometimes found positive in samples of the protected Hlawga Lake water and this test alone would not give information of any value in a comparison of these supplies In the same samples the Eijkman's test was positive in 1 c c or less indicating definite and probably recent pollution while in corresponding Hlawga Lake samples the test would not be positive in lesser quantity than 50 c c In these shallow well and surface waters also the colony characteristics did not give as definite an indication in conformity with the risks of pollution as Eijkman's test

An examination of tube-well water samples has shown that well-built wells free from any suspicion of defect will sometimes show the presence of lactose fermenters in 10 c c which would not be a satisfactory finding under European conditions Eijkman's test was found to be negative in quantities less than 100 c c except in the case of one well which was very old and under strong suspicion of defect In this well the presence of lactose fermenters in 5 c c and a positive Eijkman's test in 25 c c would direct attention to the suspicion of some degree of contamination We would suggest the standard for tube-wells in the tropics as 'Eijkman's test to be negative in quantities less than 100 c c' The results of our examinations of these water-supplies show that, fixing the standards suggested, the Eijkman's test gives information as to the occurrence of pollution closely corresponding to the degree of risk present and that a more definite opinion can be given on this one test than on the tests for lactose fermenters or types of colonies isolated and submitted to special tests

Conclusions

1 Organisms fermenting lactose at 37°C are almost universally distributed in the soil of Lower Burma in the area in which the tests were carried out Organisms fermenting glucose at 46°C were also widely distributed but to a lesser degree Both types were present in samples obtained from sources not liable to pollution of intestinal origin or even specially protected

2 Organisms giving a positive Eijkman's test have prolonged period of survival in soil under the climatic conditions prevailing These organisms are reduced in number in water more rapidly than lactose fermenters The presence of both types in equal small quantities of water would appear to be an indication of recency of pollution

3 Tests of water samples from protected sources and from sources liable to varying degrees of pollution have shown that the Eijkman's test gives an indication of the sanitary quality of the water closely in correspondence with the known risks of pollution and of more definite value than the tests for lactose fermenters at 37°C or the character of the organisms isolated and tested by means of the citrate utilization, methyl-red and Voges-Proskauer tests,

4 We suggest the following standards for waters in the tropics —

(*a*) Surface waters and shallow wells—'Eijkman's test to be negative in quantities of water less than 50 c c'

(*b*) Tube-wells—'Eijkman's test to be negative in quantities of water less than 100 c c'

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NOTES ON SOME INDIAN SPECIES OF THE GENUS *PHLEBOTOMUS*

Part XXVIII.

PHLEBOTOMUS PURII N SP

BY

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[Received for publication, November 8, 1930]

THE species here described was collected in the Darjeeling District of the Bengal Terai during August 1928 The specimens were captured in cavities in large trees in the middle of dense forest These cavities were close to the ground The insects were found in association with both sexes of *P zeylanicus* Annan and with a number of males of a species resembling *P squamirostris* Newst

Careful examination showed these specimens to be quite distinct from any of the other species of *Phlebotomus* hitherto described from Asia I have much pleasure in suggesting that this species be named *Phlebotomus purii* after Dr I M Puri, who so kindly collected the insects for me

Specimens of both sexes were received from Sukna, Tindharia and Mariabari, which are situated in the forest at the base of the Himalayan foot-hills in the Darjeeling District of Bengal

Phlebotomus purii (♀)

To the naked eye the insect is a medium-sized *Phlebotomus* of a dark brown, almost black, colour When examined under the microscope it is seen to belong to the 'recumbent-haired' group The integument of the whole body is almost black, except that the sides of the thorax are lighter, being a dark grey The hairs of the abdomen, thorax, palp, etc, are very dark brown, almost black The abdominal hairs are abundant and sleekly arranged on the dorsum of the abdomen, those on the venter are slightly ruffled The wing has a bluish iridescence, and the hairs are very dark The halteres are black.

The legs look black in some lights, while in others they have a silvery yellow appearance

In the dry state, both sexes of this species could be separated from the other species present in the collection by the arrangement of the hairs on the antennæ The majority of the antennal hairs in *Phlebotomus* project at an angle of about 45° from the antennæ (Plate LXVII, fig 10), while in this species they lie close to and almost parallel with it (Plate LXVII, fig 9)

Appearances in Stained and Mounted Specimens

The measurements of the type and four co-type females are given in Table I The type specimen and three co-types came from Sukna and the fifth from Mariandan

The *total length* of the insects was from 2 55 to 2 73 mms The shape of the cicatrices on the abdomen confirmed the recumbent character of the dorsal abdominal hairs

The *buccal cavity* (Plate LXVI fig 5) has an armature of about 20 teeth arranged in a curved row These are separated from each other and well developed The pigmented area is narrow and elongated

The *pharynx* (Plate LXVI, fig 4) is comparatively slender Its length is almost three its greatest breadth and its narrowest portion is about half the width of the broadest part The armature is inconspicuous and consists of a series of transverse ridges with serrated edges pointing posteriorly A few of the more anterior of these ridges end in slender spines The ratio palp over epipharynx averages about 3 3 The latter structure is comparatively short and stout

The *antennæ* (Plate LXVI, figs 7, 8, 9 and 10) have paired geniculate spines on segments III to XV inclusive, those on the proximal segments are comparatively short, while those on the more distal ones reach as far as the succeeding inter-segmental articulations The geniculate spines are usually difficult to distinguish because of the numerous modified hair-like scales lying parallel with the stem of the antenna (Plate LXVII, fig 9) Some of these spines show a small basal projection (Plate LXVI, fig 10) as described by Nitzulescu (1930) in the American species, *P. troglodytes* The length of the antenna is great but smaller than that of the male It is about 6 times the length of segment III and 5 times that of segments XII to XVI Segment III is very long and surpasses the end of the proboscis by about one-third of its length This segment is shorter than the combined length of segments IV and V and also that of segments XII to XVI

The *palp* (Plate LXVI, fig 2) has a formula of 1, (2, 4), 3, 5 and the relative lengths of the different segments averaged 5 1, 10 1, 13 1, 10 and 23 1 Newstead's spines are situated on the middle third of the 3rd segment and are about 40 in number

The *wing* (Plate LXVI, fig 1) is broadly lanceolate and about 3 4 times as long as broad α is almost equal to β, which is about one-fourth greater than γ The ratio δ over α is about 0 55

TABLE I

Phlebotomus pum (♀)

	Structure	Lengths in mms of specimens number — 1	2	3	4	5	Ratios, relative lengths, formulæ, etc
Body	Head and clypeus	0·370	0·376	0·385	0·385	0·385	
	Thorax	0·643	0·700	0·714	0·640	0·657	
	Abdomen proper	1·370	1·414	1·417	1·314	1·457	
	Sup clasper	0·170	0·185	0·170	0·170	0·170	
	Total length	2·55	2·67	2·73	2·51	2·67	
Mouth	Pharynx, length	0·171	0·168	0·168	0·166	0·168	= 2·7–2·9 × breadth
	Pharynx, breadth	0·060	0·063	0·060	0·057	0·057	
	Epipharynx	0·168	0·170	0·165	0·165	0·165	$\frac{P}{L}$ = 2·22–2·38 $\frac{P}{E}$ = 3·17–3·44
	Labium	0·243	0·243	0·243	0·228	0·243	
Antenna	Segment III	0·300	0·330	0·315	0·324	0·294	III < IV + V IV > V > VI
	Segment IV	0·175	0·186	0·174	0·183	0·174	III < XII–XVI
	Segment V	0·162	0·180	0·168	0·177	0·165	$\frac{2}{\text{III–XV}}$
	Segment VI	0·153	0·162	0·162	0·165	0·147	Formula
	Segs XII–XVI	0·375	0·384	0·384	0·387	0·363	
	Total length	1·870	2·000	1·943	2·000	1·790	= 6·03–6·23 × IIIrd, 4·9–5·17 × XII–XVI
Palp	Segment 1	0·048	0·048	0·045	0·042	0·048	Formula, 1, (2, 4), 3, 5
	Segment 2	0·087	0·093	0·090	0·090	0·093	Relative lengths, 5·1, 10·1, 13·1, 10, 23·1
	Segment 3	0·117	0·119	0·120	0·114	0·120	
	Segment 4	0·084	0·093	0·090	0·090	0·090	= 2nd
	Segment 5	0·214	0·213	0·195	0·186	0·228	
	Total length	0·546	0·566	0·540	0·522	0·579	
Wing	Length	2·000	2·028	2·057	2·014	2·000	= 3·33 × breadth
	Breadth	0·580	0·600	0·614	0·600	0·600	
	α	0·428	0·385	0·371	0·371	0·428	$\frac{\alpha}{\beta}$ = 0·86–1·20 $\frac{\beta}{\gamma}$ = 1·18–1·37
	β	0·357	0·385	0·430	0·414	0·385	
	γ	0·300	0·314	0·314	0·314	0·314	$\frac{\alpha}{\gamma}$ = 1·20–1·45 $\frac{\delta}{\alpha}$ = 0·48–0·68
	δ	0·257	0·200	0·185	0·178	0·250	
	ϵ	0·557	0·514	0·500	0·485	0·557	$\frac{\alpha}{\epsilon}$ = 0·74–0·77 $\frac{\theta}{\epsilon}$ = 1·82–2·05
	θ	1·014	0·970	1·014	1·000	1·014	
	τ	0·143	0·128	0·128	0·100	0·085	$\frac{\alpha+\beta}{\theta}$ = 0·78–0·80 $\frac{\text{Wing}}{\theta}$ = 1·97–2·08

TABLE I—*concld*

	Structure	Lengths in mms of specimens number —					Ratios relative lengths formulæ, e
		1	2	3	4	5	
Hind leg	Femur	0·800	0·813	0·828	0·800	0·770	$\geq$ segs 2–5
	Tibia	1·286	1·357	1·313	1·257	1·213	= 1·6 × femur, 2 × seg 1
	Tarsus, seg 1	0·628	0·670	0·657	0·628	0·643	
	Tarsus, segs 2–5	0·770	0·800	0·800	0·785	0·757	
	Total length	3·18	3·67	3·63	3·47	3·11	
	Sperm, length	0·063	0·063	0·063	0·060	0·063	= 3 × breadth
	Sperm, breadth	0·021	0·022	0·021	0·020	0·021	

The *hind leg* is much longer than the body. The femur is slightly longer than tarsal segments 2–5. The tibia is about twice the length of tarsal segment 1 and about 1·6 times that of the femur.

The *spermatheca* (Plate LXVI, fig. 3) is elongated, gradually merging into a wide duct. It is thin-walled and in some specimens it shows a slight tendency to faint crenulation at its distal end. This resembles the spermatheca of *P. squamiostris* (*cf.* Sinton, 1929, fig. 11).

The *post-genital plate* (Plate LXVI, fig. 6) carries 4 or 5 spines.

Phlebotomus puri (♂)

Specimens of this sex were obtained from Sukna, Marianbari and Tindharia.

It is a medium-sized *Phlebotomus* of the recumbent-haired group. Its general appearance is dark brown or greyish brown but is not so dark as the female. The eyes are black. The integument is brown or dark greyish brown, except for the sides of the thorax which are light grey. The dorsal abdominal hairs are recumbent, but those on the venter are inclined to be semi-erect and tufted segmentally. The hairs of the body are golden brown. The wings have a bluish golden iridescence. The legs are dark brown with silvery reflections in some lights. The antennæ are dark grey with closely applied scales. The halteres are black.

Appearances in Stained and Mounted Specimens

The measurements of the type and four co-type specimens are given in Table II. The type and three co-types were collected at Sukna, while the other co-type came from Tindharia.

The *total length* of the insect is about 2·4 to 2·9 mms.

The *buccal cavity* (Plate LXVII, fig. 8) has a small, but distinct and thin, elongated pigmented area. The buccal teeth are separated and arranged in a single curved row of about 14. The *pharynx* (Plate LXVII, fig. 2) is not

TABLE II

Phlebotomus puri (♂)

	Structure	Lengths in mms of specimens number — 1	2	3	4	5	Ratios, relative lengths, formulæ, etc
Body	Head and clypeus	0·343	0·343	0·357	0·330	0·314	
	Thorax	0·543	0·570	0·600	0·530	0·485	
	Abdomen proper	1·485	1·157	1·570	1·143	1·600	
	Sup clasper, seg 1	0·414	0·426	0·408	0·396	0·414	
	Total length	2·8	2·8	2·93	2·4	2·8	
Mouth	Pharynx, length	0·150	0·147	0·144	0·147	0·150	= 3·12–3·3 × breadth
	Pharynx, breadth	0·048	0·045	0·045	0·041	0·045	
	Epipharynx	0·150	0·156	0·150	0·150	0·153	$\frac{P}{L}$ = 2·40–2·45 $\frac{P}{E}$ = 3·2–3·33
	Labium	0·200	0·207	0·200	0·200		
Antenna	Segment III	0·450	0·435	0·460	0·444	0·432	III < IV + V IV > V > VI
	Segment IV	0·288	0·285	0·273	0·285	0·276	III > XII–XVI
	Segment V	0·282	0·270	0·273	0·270	0·276	Formula $\frac{I}{III–XV}$
	Segment VI	0·255	0·255	0·253	0·246	0·273	
	Segs XII–XVI	0·360	0·360	0·357		0·357	
	Total length	2·643	2·600	2·714		2·570	= 5·90–5·97 × IIIrd, 7·22–7·34 × XII–XVI
Palp	Segment 1	0·033	0·036	0·030	0·030	0·036	Formula, 1, (2, 4), 3, 5
	Segment 2	0·075	0·081	0·084	0·075	0·081	Relative lengths, 4, 10, 13, 10, 25
	Segment 3	0·100	0·105	0·105	0·100	0·102	
	Segment 4	0·075	0·081	0·087	0·075		= 2nd
	Segment 5	0·198	0·210	0·180	0·200		
	Total length	0·481	0·513	0·486	0·480		
Wing	Length	1·714	1·730	1·770	1·630	1·714	= 4 × breadth
	Breadth	0·428	0·428	0·443	0·400	0·420	
	α	0·243	0·314	0·343	0·270	0·300	$\frac{\alpha}{\beta}$ = 0·61–1·0 $\frac{\beta}{\gamma}$ = 1·10–1·47
	β	0·400	0·343	0·343	0·330	0·357	
	γ	0·270	0·270	0·285	0·300	0·278	$\frac{\alpha}{\gamma}$ = 0·9–1·2 $\frac{\delta}{\alpha}$ = 0·3–0·46
	δ	0·071	0·130	0·157	0·107	0·114	
	ϵ	0·335	0·413	0·457	0·370	0·400	$\frac{\alpha}{\epsilon}$ = 0·72–0·75 $\frac{\theta}{\epsilon}$ = 1·94–2·43
	θ	0·814	0·857	0·885	0·770	0·843	$\frac{\alpha+\beta}{\theta}$ = 0·75–0·79 $\frac{\text{Wing}}{\theta}$ = 2·0–2·11
	τ	0·128	0·085	0·071	0·114	0·071	

P squamiostris has a pharyngeal armature with well-developed teeth, the proximal segment of the superior clasper is stout and wide, and the non-deciduous hair on the distal segment is proximal to the proximal pair of spines

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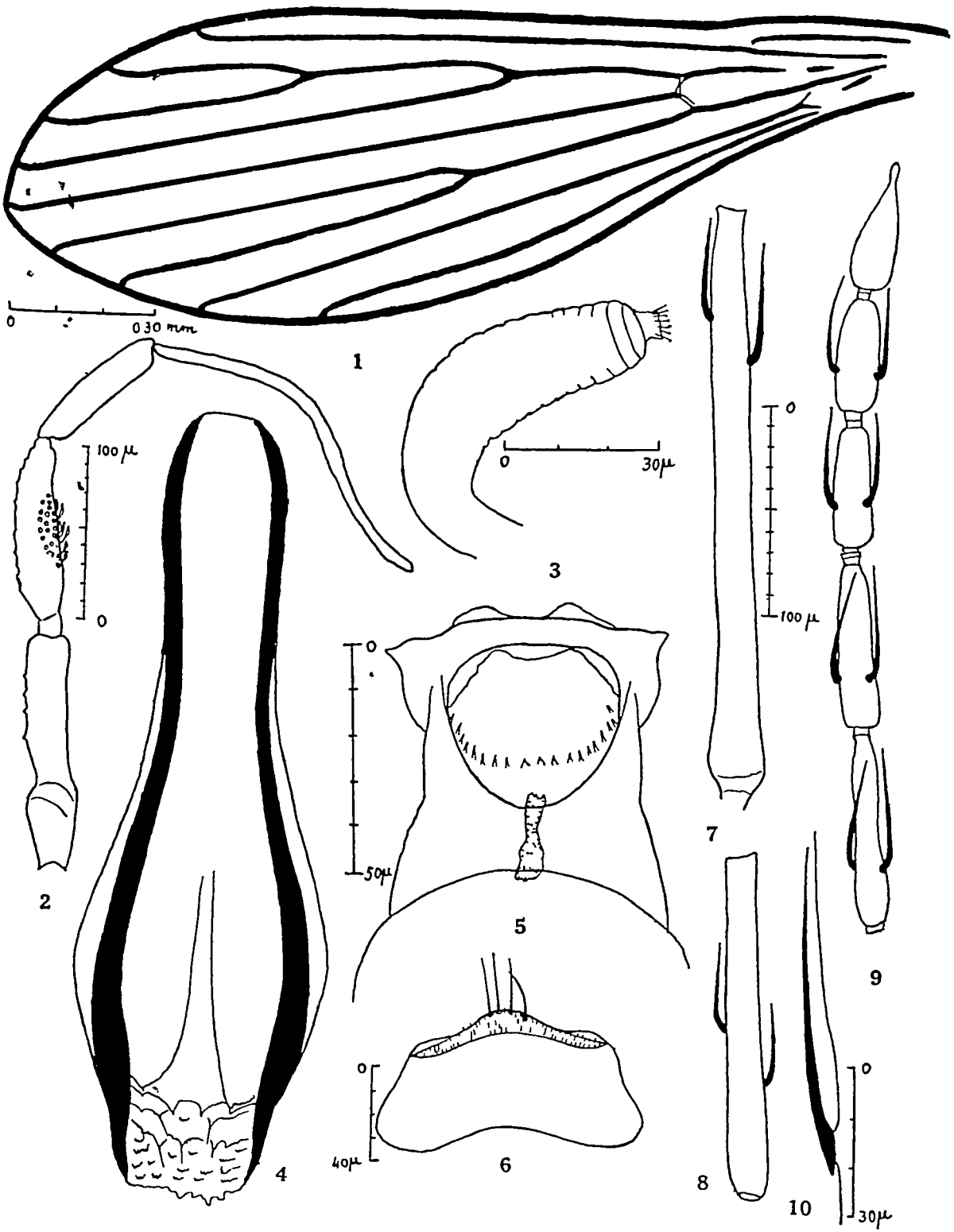
EXPLANATION OF PLATE LXVI

Phlebotomus puri (♀)

Fig 1 Wing
„ 2 Palp
„ 3 Spermatheca
„ 4 Pharynx
„ 5 Buccal cavity
„ 6 Post-genital plate
„ 7 Segment III of antenna
„ 8 Segment IV of antenna
„ 9 Segments XII to XVI of antenna
„ 10 Geniculate spine on segment VI of antenna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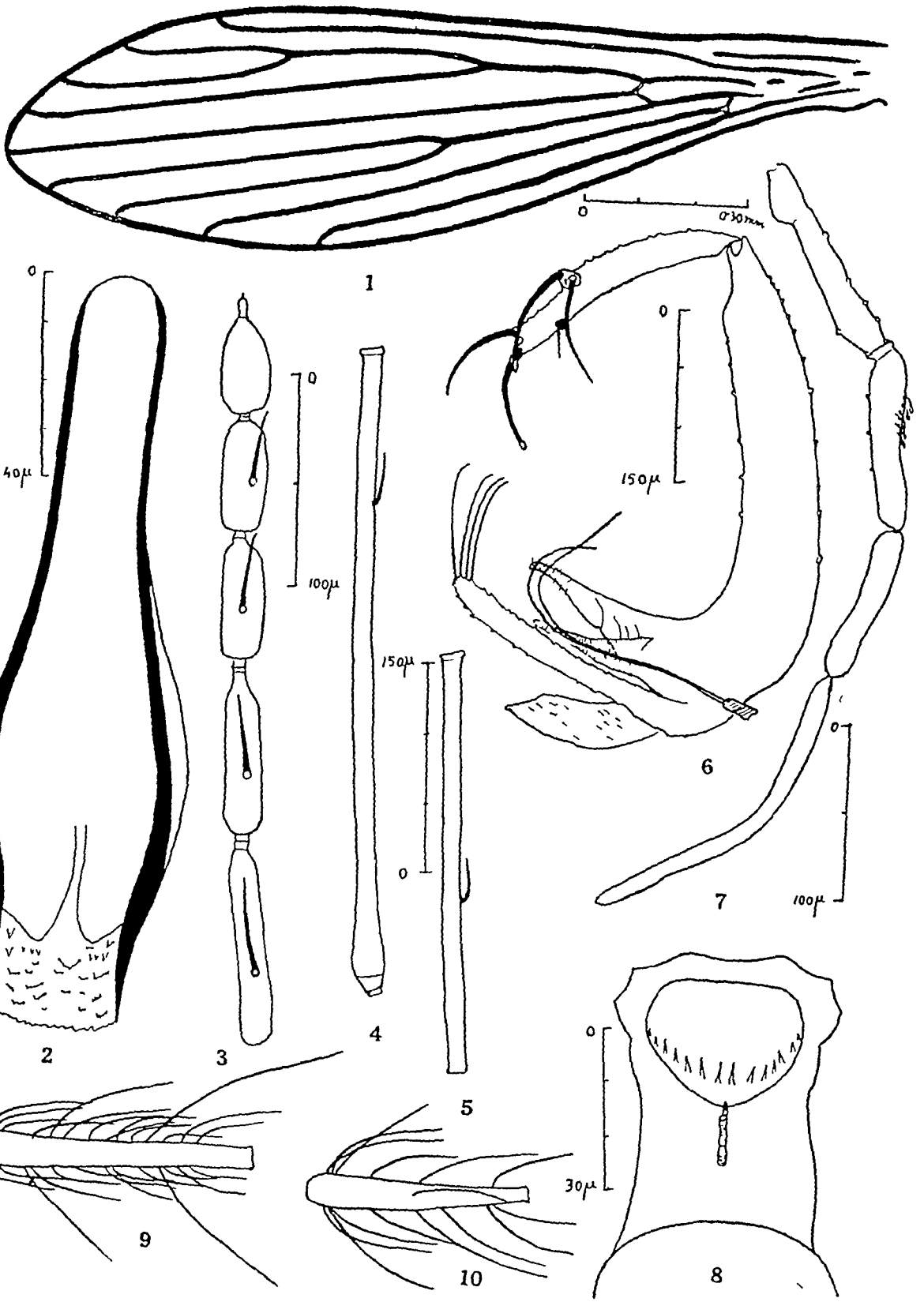
Explanation of Plate LXVII

Phlebotomus puri (♂)

Fig 1 Wing
„ 2 Pharynx
„ 3 Segments XII to XVI of antenna
„ 4 Segment III of antenna
„ 5 Segment IV of antenna
„ 6 Male hypopygium
„ 7 Palp
„ 8 Buccal cavity
„ 9 Segment of antenna showing arrangement of hairs in *P puri* (♀)
„ 10 Segment of antenna showing arrangement of hairs in *P zeylanicus* (♀)

PLATE LXVII



THE EFFECT OF DILUTION ON THE PROPERTIES OF AN ANTISEPTIC

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IT is a well-known fact that with increasing concentration of a poison in solution, its lethal effects are progressively enhanced In the case of those poisons, however, which dissociate in solution, an increase in the concentration of the poison in solution is accompanied by a diminution in the dissociation of the poison And if the dissociated molecules of the poison also possess some lethal properties, it is obvious that while the poison in concentrated solution may tend to become more lethal due to its increased combination with the tissues, its diminished dissociation will tend to lower its effectiveness These considerations led one of the authors to express the view (Seth, 1923) that for a given set of conditions, there must be a particular concentration at which the resultant of these two antagonistic factors reaches a maximum, and at which, therefore, the poison is most effective

In a recent investigation (Seth, 1930) the above hypothesis has been verified experimentally, and it has been found that up to a certain point, an

increase in the dilution of a poison, which dissociates in solution, causes a steady decline in its lethal properties Beyond this point, however, there is a limited region of renewed potency in which further dilution of the poison definitely enhances its lethal effects Still further dilution, once again decreases the effectiveness of the poison

Since the action of poisons, antiseptics and disinfectants has essentially a common basis, it was considered worth while to extend the work to antiseptics and disinfectants with a view to determine if the results recorded in the case of poisoning of tadpoles (Seth, 1930) could be verified by the use of an entirely different technique and a totally different type of experimental organism, and in view of such findings, if any economy could be effected in the use of antiseptics and disinfectants

This paper embodies the results obtained with antiseptics

Experimental

Mercuric chloride, carbolic acid and ethyl alcohol were the three antiseptics employed in varying dilutions, the exact range being determined by preliminary experiments The relative power of the various solutions, in preventing the growth of *Staphylococcus aureus,* was taken as a measure of their effectiveness

0 8 c c of the antiseptic solution of each dilution was measured out into small sterile test tubes, to each of which 0 2 c c of the bacterial emulsion in distilled water (337 million organisms per c c) was then added and mixed, the exact time of such addition being noted with the help of a stop watch At intervals of one minute, a loopful (diameter of the platinum loop being 3 mm) of the contents of each tube was inoculated on an agar slant * This was continued for six minutes All the tubes were then incubated for 48 hours at 37°C, after which the relative growth of the organism in each tube was recorded, the following notation being used —

Profuse growth	=	+++
Moderate growth	=	++
Poor growth (a few isolated colonies)	=	+
No growth	=	—

* As it was important for purposes of this investigation to observe the relative growth of the organism in the different dilutions of an antiseptic solution, agar slants were used in preference to broth solutions, even though it was realized that the use of the former introduced an experimental error due to the continued intimate contact between the antiseptic and the organism for some time after their transference to the surface of the agar slant However, as this source of error was common to all the experiments performed, the results are strictly comparable, even if they are not absolute

The results are expressed below in Tables I, II and III

TABLE I

Mercuric chloride

No	Conc of $HgCl_2$ solutions (gramme $HgCl_2$ per litre)	GROWTH OF THE ORGANISM AFTER TREATMENT WITH THE ANTISEPTIC, FOR ONE TO SIX MINUTES					
		1 min	2 mins	3 mins	4 mins	5 mins	6 mins
1	0·009	+++	+++	+++	+	+	—
2	0·008	+++	+++	+++	+++	+++	+++
3	0·007	+++	+++	+++	++	—	—
4	0·006	+++	+++	+++	+++	+	—
5	0·005	+++	+++	+++	+++	++	+
6	0·004	+++	+++	+++	+++	+++	++
7	0·003	+++	+++	+++	+++	+++	+++
8	0·002	+++	+++	+++	+++	+++	+++
9	0·001	+++	+++	+++	+++	+++	+++
10	0·000	+++	+++	+++	+++	+++	+++

It will be noticed that solution No 3 is more effective than either No 2 or No 4

TABLE II

Carbolic acid

No	Conc of solutions (gramme carbolic acid per cent)	GROWTH OF THE ORGANISM AFTER TREATMENT WITH THE ANTISEPTIC, FOR ONE TO SIX MINUTES					
		1 min	2 mins	3 mins	4 mins	5 mins	6 mins
1	1·6	—	—	—	—	—	—
2	1·5	+	—	—	—	—	—
3	1·4	++	++	++	++	++	++
4	1·3	++	+	—	—	—	—
5	1·2	++	++	++	+	+	—
6	1·1	++	++	++	++	+	+

The above figures very clearly demonstrate the phenomenon under discussion The effectiveness of the antiseptic declines with progressive dilution

of the solution up to a certain point, beyond which further dilution causes a phenomenal recovery in the activity of the antiseptic, as indicated in solution No 4, and to a lesser extent in solutions Nos 5 and 6

TABLE III

Ethyl alcohol

No	Conc of solutions (c c absolute alcohol per 100 c c solution)	GROWTH OF THE ORGANISM AFTER TREATMENT WITH THE ANTISEPTIC, FOR ONE TO SIX MINUTES					
		1 min	2 mins	3 mins	4 mins	5 mins	6 mins
1	40	++	—	—	—	—	—
2	38	+	—	—	—	—	—
3	36	++	—	—	—	—	—
4	34	++	++	+ (7)	+ (1)	+ (2)	—
5	32	+++	++	+ (1)	—	—	—
6	30	+++	+++	++	+ (1)	—	—

Figures within brackets (Table III) represent the number of colonies produced They are presented here to illustrate the comparative effectiveness of alcohol in those cases where the differences in growth of the organism are not large enough to be represented adequately by mere plus and minus signs An analysis of these figures shows clearly that although ethyl alcohol also recovers its activity in the vicinity of a concentration of 32 per cent, the recovery is not so marked as in the case of mercuric chloride or carbolic acid An explanation for this difference is given below

DISCUSSION OF RESULTS

In agreement with the observations previously made in the case of poisons (Seth, 1930), the results recorded in this paper show that up to a certain point an increase in the dilution of the antiseptic solution causes a steady decline in its antiseptic properties, and that beyond this point there is a limited region of renewed potency in which further dilution of the solution definitely enhances its antiseptic value Still further dilution, however, once again reduces the effectiveness of the antiseptic The exact range of concentrations where this phenomenon manifests itself, naturally varies with the antiseptic used, all other factors remaining constant

It has been noted, however, that although there are two prominent regions of activity for each antiseptic, the maximum effect of the antiseptic in the 'dilute region' never attains the level of the maximum effect in the

'concentrated region' For example, a concentration of 0 007 g $HgCl_2$ per litre is more effective than either 0 008 g or 0 006 g $HgCl_2$ per litre, but it is much less effective than a concentration of say 0 01 g $HgCl_2$ per litre (*see* Table I)

In explanation of the above observations it is suggested that the antiseptic action of substances which dissociate in solution, depends on two factors, viz,

Factor A —The influence of the undissociated molecules of the antiseptic on the organism

Factor B —The influence of the ions of the antiseptic on the organism

At higher concentrations of the antiseptic, therefore, Factor A predominates With increase in dilution, the number of undissociated molecules of the antiseptic diminishes, and as long as the number of ions is not enough to counterbalance the decline in Factor A, the effectiveness of the antiseptic as a whole steadily decreases Beyond a certain point, however, the action of the antiseptic is once again intensified due to such an increase in Factor B, as more than counterbalances the decline in Factor A

From what has been stated above it also follows that in the case of those antiseptics which dissociate very feebly in solution, Factor A would be operative practically all through and, therefore, the zone of high activity in the 'dilute region' would not be very prominent This was found to be the case with ethyl alcohol, where it was noticed (*see* Table III) that although 32 per cent alcohol was more effective as an antiseptic than either 34 per cent or 30 per cent alcohol, the difference in the activity of the various solutions was not as marked as in the case of mercuric chloride and carbolic acid

The findings recorded here furnish an independent verification of the observations made with poisons (Seth, 1930) However, they can have no practical application in effecting a more economic use of antiseptics, since a very rapid destruction of all the pathogenic organisms is required, and it has to be obtained by the use of fairly strong solutions of antiseptics, in spite of the relatively higher cost Circumstances are entirely different, however, in the case of disinfectants, for the organisms sought to be destroyed, need not necessarily be killed instantly, and therefore the use of more dilute solutions can not materially affect the efficiency of the disinfectants Moreover, disinfection of wells and water tanks, etc, involves the use of fairly large quantities of disinfectants and consequently even a small difference in the concentration of the disinfectant used would effect appreciable economy Work in this direction is in progress and the results will be reported later

SUMMARY

Up to a certain point, an increase in the dilution of an antiseptic which dissociates in solution causes a steady decline in its activity Beyond this point there is a limited range in which further dilution definitely increases the relative effectiveness of the antiseptic Still further dilution, however, once again lowers the activity of the antiseptic

In explanation of this phenomenon, it is suggested that the activity of an antiseptic which dissociates in solution depends, amongst other things, on the influence of the ions as well as of the undissociated molecules of the antiseptic on protoplasm The relative influence of each of these two factors varies with the dilution of the solution, there being a zone of high activity in the 'concentrated region,' due mainly to the influence of the undissociated molecules, and another region of high activity in the 'dilute region,' due largely to the influence of the ions in solution

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STUDIES IN THE NUTRITIVE VALUE OF INDIAN VEGETABLE FOOD-STUFFS

Part I

NUTRITIVE VALUES OF PIGEON PEA (*CAJANUS INDICUS*) AND FIELD PEA (*PISUM ARVENSE*, LINN)

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THE present investigation is an attempt at a scientific study of some of the more important Indian vegetable food-stuffs, as to the nature of their proteins and their relative value in nutrition The commoner Indian pulses have been chosen for the present work as they form the main source of protein in the Indian diet Being cheap, they are popularly regarded as desirable substitutes for the more expensive animal proteins It is of importance therefore to ascertain whether the proteins are good in quality and, if so, how far they supply the essential amino acids required for growth and maintenance This part (Part I) deals with the two Indian pulses, Pigeon pea (*Cajanus indicus*) and Field pea (*Pisum arvense*)

Pigeon pea is extensively cultivated in all tropical countries on account of the green pea it affords, being an excellent substitute for the common garden pea, especially as it comes into season during the hot months when the ordinary pea is not available In India it is most frequently grown as a mixed crop or a rotation crop for cereals According to the Season and Crop Report, the area under this crop in the presidency of Bombay is 443,365 acres The

pulse is highly esteemed throughout India and enters very largely into the vegetarian diet of the people It is sold in the form of split peas In Northern Bengal and Assam, the pulse is specially grown as a host plant for the lac insect The most common vernacular names of this pulse are Dal, Tuar, Tur, Arhar, Togari, Tovare, Kandipappu, etc

The field pea or the grey pea is cultivated as a *rabi* crop in many parts of India during the cold weather in the same way as the common yellow coloured garden pea (*Pisum sativum*) It produces small, round compressed, greenish or marbled grey seeds, which are eaten as dal In many localities, the green pods are collected while the plant is growing and the young seeds cooked and eaten The green plant is extensively used and valued as fodder, more especially in the Punjab and Bombay, where they are regarded as equal to hay The field pea is known by the following vernacular names Hirwa Vatana, Mattar, Kulon, Koirani, etc

The nutritional value of pulses depends largely on the proteins they supply and the investigations of Osborne and others have shown that there is a definite relation between the chemical composition of a protein and its nutritional efficiency The major part–nearly 80 per cent–of the proteins of leguminous seeds belong to the class of globulins, the rest being made up of albumins, polypeptides and free amino acids To get an insight into the nature of the proteins of these pulses their total globulins were isolated in a pure condition and analysed for their constituent amino acids The biological values of these pure globulins as well as of the total proteins in the whole seed were determined at approximately 5 per cent level of intake by means of feeding experiments on albino rats

There are at present available two methods of estimating the biological values of proteins One of them originally introduced by Thomas (1909) is based on the estimation of the nitrogen balance and is now known in its improved form as the method of Mitchell (1924) (also Mitchell and Carman, 1926) The latter involves a study of the nitrogen metabolism of young growing rats under controlled conditions of feeding and the nutritive value of a protein is taken as the percentage of absorbed nitrogen that is retained in the experimental animal and is utilized for growth and constructive processes in the body This percentage is also known as the Biological Value of the protein, a term first introduced by Thomas

In the alternative method introduced by Osborne, Mendel and Ferry (1919), the nutritive value of a protein is measured by the gain in body-weight of the experimental animal per gramme of protein consumed The two methods are not strictly comparable as the one measures the efficiency of a protein to make good over short periods of time the wear and tear of body-tissues, while the other measures the ability of a protein to build new tissues It is not possible to say which method gives a more accurate estimate of the nutritive value of a protein, but in the present work Mitchell's method has been adopted, as the experimental periods are of shorter duration and the protein food required for

the metabolism experiments is much less for the same number of rats than in Osborne's method

EXPERIMENTAL A

Isolation and analysis of the globulins

Material

The varieties of the two pulses, pigeon pea and field pea, obtainable in the local market, were sun dried, decorticated and then ground to flour to pass a sieve of 60 mesh Specimens of the flours dried at 100°C gave the following percentages on analysis —

Table I

Pulse	Ash	Crude fibre	Ether extractives	Crude protein (N × 6·25)	Carbohydrates by difference	True protein determined separately
Cajanus indicus	3·99	1·16	2·44	25·63	67·38	22·14
Pisum arvense	2·75	1·98	1·45	25·57	69·15	23·31

Preparation of the globulins

Extraction —Preliminary trials showed that it is advantageous to use a fairly high concentration of NaCl solution to extract the globulins It prevents, during extraction and filtration, the action of the oxidases and peroxidases such as tyrosinase, etc , which are usually present in the seeds About 1 kilo of the flour was in each case treated with 5 to 6 litres of 10 per cent NaCl solution and stirred mechanically for 3 to 4 hours The mixture in each case was strained through cheese cloth and the liquid poured into large fluted gravity filters and allowed to stand overnight, toluene being added to prevent bacterial action About 2 to 2·5 litres of slightly coloured opalescent extracts were thus obtained

Precipitation —The globulins were precipitated from the extract by the following two methods —

(1) Dialysis —The extracts were dialysed against cold, running distilled water for 6 to 8 days until the dialysates were free from traces of chlorides The globulins which were precipitated were centrifuged off, redissolved in 5 per cent NaCl solution, filtered and dialysed as before Toluene was added to prevent bacterial action

(2) Dilution and acidification —The extracts were diluted 5 to 6 times with distilled water till cloudiness appeared and then saturated with carbon dioxide A few drops of acetic acid were then added to complete the precipitation of the globulin

Purification —The precipitates of the globulins, obtained as above, were washed several times with distilled water by decantation They were then,

centrifuged off and dehydrated by washing with graded strengths of alcohol and finally by ether The ether was driven off and the preparations were powdered and passed through a 120 mesh sieve preparatory to further examination These powders represent the total globulins in each seed and no attempt was made to separate the two or more globulins each of them may contain (Sundaram, Norris and Subrahmanyan, 1929)

General properties of the globulins

The preparations were light powders of cream white colour All of them gave colour reactions characteristic of proteins and contained sulphur, tyrosine, tryptophane They were soluble in dilute alkali and glacial acetic acid On analysis they gave the following percentages —

TABLE II

	GLOBULIN FROM			
	Cajanus indicus		*Pisum arvense*	
	Dialysis	Dilution	Dialysis	Dilution
Moisture	8·19	7 88	9 71	9·23
Ash	0·95	0·87	0·83	0·89
	On ash and moisture free basis			
Nitrogen	15·82	15 72	16 45	16·44
* Sulphur	0·472	0·470	0 312	0·306

* Hoffman and Gortner, 1923

Analysis—The nitrogen distribution was determined by the method of Van Slyke as modified by Plimmer and Rosedale (1925*a*) Several factors have been reported as influencing the figures obtained as nitrogen distribution numbers of proteins (Knaggs, 1923, Thimman, 1926, Daft, 1929) To obtain comparable results, these factors were, as far as possible, kept constant throughout this series of experiments Immediately after hydrolysis, the insoluble melanin was filtered off and its nitrogen separately estimated The amount of CaO used for distillation of ammonia was 3 5 gs for every 1,000 mg of nitrogen in the hydrolysate This quantity was calculated to be more than sufficient to neutralize the slight acidity of the hydrolysate and also to displace the maximum amount of ammonia that may be present in proteins The volume in which the precipitation of the diamino acids was allowed to take place, was 200 c c containing 350 to 360 mg of nitrogen and enough HCl to make it one normal The solution was heated to boiling, prior to the addition of 15 gs of phosphotungstic acid after which it was allowed to stand for

24 hours at room temperature and anothei 24 hours at about 8° to 10°C When the phosphotungstic acid was added in the cold, it was found impossible to redissolve the precipitate formed, by heating For the rest, the procedure followed was similar to that of Plimmer and Rosedale (1925*a*)

Arginine in the diamino fraction was estimated by the method of Plimmer (1916) Sulphur was determined according to Plimmer and Lowndes (1927)

Free amino nitrogen in the native proteins was estimated in a one per cent solution of the globulins in dilute alkali The micro Van Slyke apparatus was used and the time allowed for reaction was 30 minutes

The results of the foregoing determinations are given below —

TABLE III

(*Expressed as percentages of total nitrogen*)

Form of nitrogen	GLOBULINS OF			
	Cajanus indicus		*Pisum arvense*	
	Dialysis	Dilution	Dialysis	Dilution
Acid insoluble melanin	0·96	0 62	0·62	0·40
Acid soluble melanin (adsorbed by lime)	0·57	0·57	1 58	1 98
Amide	9·98	10·27	10·92	10·28
Diamino —				
Arginine	11 88	11 98	18·44	18·74
Histidine	4 81	4 01	3·18	3 06
Cystine	0 50	0·42	0·20	0·24
Lysine	8·01	9·11	9·36	9·74
Mono amino —				
Amino	60·99	61 82	50·88	50·30
Non-amino	2·88	2·01	4 76	5 04
TOTAL	100·58	100·81	99 94	99 78
Free amino nitrogen in the native proteins				
By direct estimation	4 90	5·32	4 24	4 92
Half lysine nitrogen	4 01	4 55	4 68	4 87

According to Plummer and Rosedale (1925*b*), arginine is not completely precipitated by phosphotungstic acid and part of it comes down in the mono amino fraction It was therefore estimated directly in the hydrolysate

Tyrosine was estimated by two methods —

(1) Zuwerkalao (1926) and (2) Folin and Ciocalteu (1927) and tryptophane by the method of Tillmans and Alt (1925)

The figures obtained for cystine as a result of Van Slyke analysis cannot be taken as correct, as during hydrolysis, the cystine is partially racemized and incompletely precipitated by phosphotungstic acid Independent determinations of cystine were therefore carried out by the method of Folin and Looney (1922)

The foregoing estimations were carried out on mixed samples of the preparations and the results are given below —

TABLE IV

(*Expressed as per cent of protein-ash and moisture free*)

Amino acid	Globulins of		Method
	Cajanus indicus	*Pisum arvense*	
Lysine	7·03	8·19	Van Slyke
Histidine	2·56	1 90	Do
Arginine	5 84	9 50	Do
Do	6 91	10·64	Direct estimation
Cystine	1 58	1 12	Folin and Looney
Tyrosine	3 12	1 98	Zuwerkalao
Do	3 35	2·32	Folin and Ciocalteu
Tryptophane	0·46	0·51	Tillmans and Alt

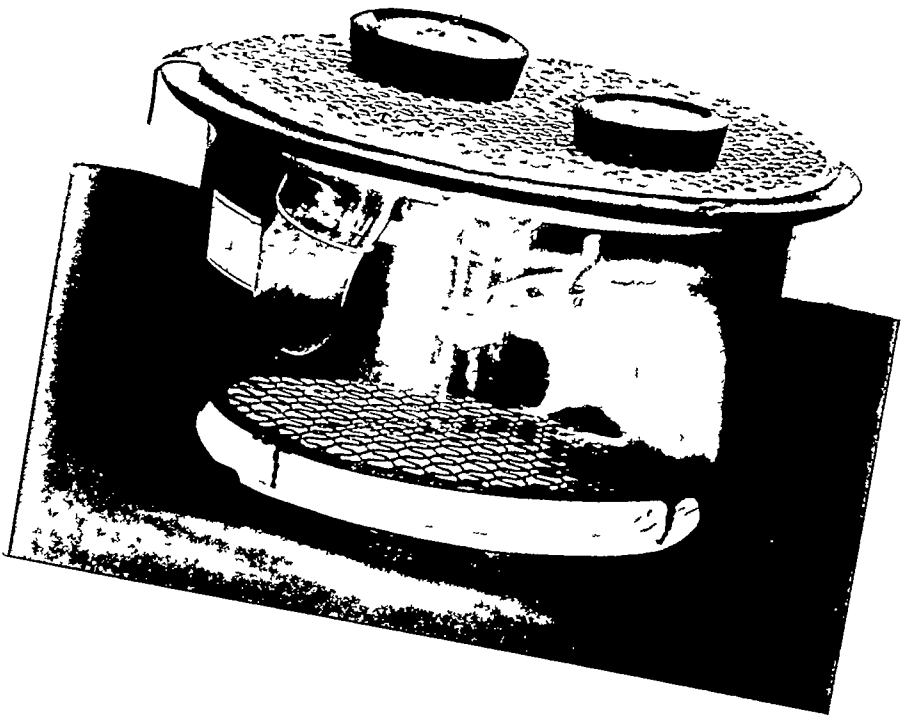
EXPERIMENTAL B

FEEDING EXPERIMENTS

Two sets of young albino rats, each set belonging to the same litter, were placed in large circular glass jars, measuring 8 in in diameter and 9 in in depth Food and water were given in glass vessels suspended from the sides of the jars Weighted wire mesh covers were placed on top and all precautions were taken to ensure sufficient ventilation On the bottom of the jars, filter papers cut to fit in exactly, were placed for the absorption of the urine The rats were supported about 2 in above the filter papers on a circular disc of 1/3 in mesh wire netting, resting on wire legs By this arrangement consumption of fæces and filter paper was entirely avoided (*vide* Plate LXVIII)

In all the following experiments the metabolic periods were of ten days' duration, during the last seven days of which the urine and fæces were collected daily In changing from one ration to another, three days were allowed

PLATE LXVIII



to elapse before urine and fæces were again collected Body-weights were taken at the beginning and end of each balance period

Urine and fæces were collected daily The fæces were preserved in alcohol acidulated with sulphuric acid The jar, the wire net support and the filter papers of each rat were thoroughly washed every day with hot dilute sulphuric acid The washings–about 250 c c–were filtered through a Buchner funnel and placed in 250 c c measuring flasks The next day they were made up to volume at room temperature and poured into two litre bottles in which the weekly composite samples were kept Aliquots of 200 c c were analysed for total nitrogen

The week's collection of fæces were digested by the Kjeldahl method and the residue made up to volume, aliquots of which were used for nitrogen estimation

The rations were prepared to contain approximately 5 per cent of protein Their percentage composition is given below —

TABLE V

	Ration		
	Non-protein	Globulin	Pulse flour
Cane sugar	10	10	10
Butter fat	8	8	8
Cod-liver oil	2	2	2
Agar agar	1	1	
*Salt mixture	4	4	3
Globulin		5	
Flour			Enough to contain 5 per cent protein (N × 6 25)
Starch	75	70	To make up to 100

*Osborne and Mendel (1920)

All the ingredients of a ration except the fats were mixed with sufficient water and cooked on a bath until the starch was thoroughly dextrinized The fats were then mixed in, the mixture spread out in thin layers on glass plates and dried at about 60° to 70°C It was then broken up, ground to powder and analysed for total nitrogen The food was weighed out each day and mixed with water to a thick consistency to prevent scattering The daily residues of food left uneaten were collected, dried and subtracted to obtain the average daily intake

To maintain the appetite of rats throughout the experimental periods, each rat was fed daily, apart from its food, with 50 mg of dried brewer's yeast containing 2 9 mg nitrogen and 3 to 5 drops of cod-liver oil The nitrogen of the yeast is not taken into account in calculating the metabolism data

The results of the metabolic experiments are given in Tables VI and VII The figures for the intake of food and nitrogen, and fæcal and urinary nitrogen

TABLE VI

Metabolism data

Biological values of casein and globulins of Pisum arvense *and* Cajanus indicus

Rat number	Initial weight g	Final weight g	Food intake g	Nitrogen intake mg	Faecal nitrogen mg	Urinary nitrogen mg	Metabolic nitrogen in faeces per gramme of food mg	Endogenous nitrogen in urine per 100 g body-weight mg	Food nitrogen in faeces mg	Absorbed nitrogen mg	Food nitrogen in urine mg	Total food nitrogen retained mg	Biological value Per cent	Average biological value Per cent
Period 1, Protein free ration N = 0·11 per cent														
1	70·0	66·0	4·45	4·96	10·74	14·30	2·41	21·03						
2	78·5	73·0	4·69	5·23	11·15	16·70	2·38	22·05						
3	60·5	57·1	3·81	4·24	8·66	14·66	2·27	21·96						
4	74·0	69·0	4·14	4·61	9·98	15·00	2·41	20·98						
5	80·0	76·5	5·16	5·75	10·80	15·93	2·09	20·35						
6	59·2	55·5	3·72	4·14	9·14	12·00	2·46	20·92						
Period 2, Casein ration, N = 0·71 per cent														
1	69·0	72·5	5·09	35·35	16·61	16·73			3·43	31·92	2·41	29·51	92	89
2	75·8	78·8	5·28	36·67	13·57	19·27			0·00	36·67	2·37	34·30	94	
3	58·5	60·8	4·07	28·27	9·97	16·86			0·00	28·27	2·43	25·84	91	
4	72·5	72·2	4·11	28·54	10·87	17·31			0·27	28·27	2·48	25·79	91	
5	76·8	78·7	5·26	36·54	12·47	22·40			0·00	36·54	5·71	30·83	84	
6	57·0	58·5	3·86	26·81	9·56	16·14			0·00	26·81	4·72	22·09	82	

Period 3, Globulin (*Cajanus indicus*) ration, N = 0·91 per cent

1	71·7	71·0	3·94	35·65	16·76	24·93			5·85	29·81	11·05	18·76	63	
2	76·5	76·1	4·19	37·91	16·60	27·16			4·45	33·46	10·65	22·81	68	
3	61·5	59·0	3·04	27·51	11·15	21·15			1·75	25·76	7·06	18·70	73	
4	71·5	71·7	3·87	35·01	13·23	23·91			2·59	32·42	9·63	22·79	70	69
5	80·2	79·8	3·65	33·03	14·90	25·50			2·60	30·13	7·46	22·97	76	
6	58·2	58·5	3·43	31·03	9·42	21·70			0·78	30·25	10·85	19·40	64	

Period 4, Globulin (*Pisum arvense*) ration, N = 0·98 per cent

1	68·9	65·3	2·83	27·73	12·19	25·63			3·84	23·89	13·12	10·77	45	
2	76·3	76·0	3·40	33·32	14·83	30·00			4·09	29·23	13·67	15·56	53	
3	59·0	54·8	2·13	20·86	13·14	20·79			5·68	15·18	7·96	7·22	48	
4	71·8	69·3	3·07	30·07	14·13	26·09			5·17	24·90	12·34	12·56	50	48
5	77·1	72·6	2·49	24·40	14·94	27·59			4·96	19·44	9·88	9·56	49	
6	58·1	55·5	2·43	23·81	8·10	21·93			1·90	21·91	12·05	9·86	45	

Period 5, Protein free ration, N = 0·11 per cent

1	64·0	57·3	3·14	3·48	9·77	10·91	3·11	17·86						
2	75·1	68·5	2·82	3·13	9·59	15·24	3·40	21·22						
3	53·7	50·8	2·59	2·87	10·08	11·36	3·89	21·74						
4	69·3	69·0	2·80	3·12	8·65	13·15	3·09	18·98						
5	70·5	65·2	2·27	2·57	10·58	16·79	4·66	24·75						
6	53·7	52·0	2·98	3·33	7·69	8·57	2·58	16·22						

TABLE VII

Metabolism data

Biological values of the total proteins in the pulses Cajanus indicus *and* Pisum arvense

Rat number	Initial weight g	Final weight g	Food intake g	Nitrogen intake mg	Faecal nitrogen mg	Urinary nitrogen mg	Metabolic nitrogen in faeces per gramme of food mg	Endogenous nitrogen in urine per 100 g body-weight mg	Food nitrogen in faeces mg	Absorbed nitrogen mg	Food nitrogen in urine mg	Total food nitrogen retained mg	Biological value Per cent	Average biological value Per cent
Period 1, Non-protein ration, N = 0·093 per cent														
7	96·5	96·0	6·25	5·78	15·17	16·63	2·43	17·28						
8	120·5	117·0	6·06	5·60	19·23	15·28	3·17	12·85						
9	110·5	108·5	7·70	7·12	18·90	13·92	2·45	12·71						
10	94·5	94·3	6·45	5·96	15·84	14·26	2·46	15·10						
11	95·0	93·0	7·33	6·78	18·45	16·16	2·52	17·52						
12	71·7	69·5	4·36	4·03	11·99	10·69	2·75	15·15						
Period 2, *Pisum arvense* flour ration, N = 0·962 per cent														
7	102·8	102·0	6·12	58·89	28·97	31·67			11·44	47·45	14·24	33·21	70	
8	121·1	115·0	5·35	51·47	25·80	30·38			6·95	14·52	11·59	29·95	67	
9	115·5	111·7	6·17	59·37	32·25	27·84			15·42	43·95	12·16	31·79	72	69
10	98·5	97·5	5·42	52·16	25·34	28·37			9·02	43·14	12·87	30·27	70	
11	98·4	95·5	5·52	53·11	28·29	31·63			8·91	44·20	14·73	29·47	67	
12	72·5	69·2	3·82	36·76	17·65	19·54			6·28	30·48	8·56	21·92	72	

Period 3, *Cajanus indicus* flour ration, N = 0·852 per cent

7	104·0	100·5	5·18	44·10	30·77	24·78			13·67	30·43	7·64	22·79	75	
8	118·7	115·4	5·63	47·93	29·53	25·50			7·73	40·20	9·21	30·99	77	
9	114·0	112·0	6·08	51·76	36·23	24·14			17·99	33·77	7·31	26·46	78	79
10	98·5	98·5	5·52	46·99	26·48	24·11			6·79	40·20	7·83	32·37	81	
11	96·8	94·5	4·77	40·61	28·52	21·73			7·02	33·59	5·15	28·44	85	
12	72·7	70·1	3·80	32·35	19·18	15·82			7·02	25·33	4·50	20·83	82	

Period 4, Non-protein ration, N = 0·093 per cent

7	101·8	93·8	2·45	2·27	9·15	16·13	3·74	16·49						
8	114·2	110·2	3·94	3·64	16·63	16·23	4·22	14·46						
9	113·6	106·5	3·63	3·36	11·88	17·58	3·27	15·97						
10	98·0	92·1	3·21	2·97	13·24	16·40	4·12	17·24						
11	95·0	87·5	2·57	2·37	14·15	15·72	5·50	17·22						
12	70·1	63·1	3·04	2·91	10·41	10·79	3·43	16·21						

represent daily averages The variations in the endogenous nitrogen and the metabolic nitrogen in the periods intervening between the first and final periods of non-protein feeding are taken to be linear and calculated respectively from the average body-weight of the experimental animals and the amounts of food they consumed

Discussion

The figures for the distribution of nitrogen show that the different preparations of each one of the globulins are identical in composition Arginine as estimated in the protein is slightly higher than when estimated in the diamino fraction, showing thereby that the amino acid is not completely precipitated by phosphotungstic acid

The free amino nitrogen of both the globulins do not very much differ from half their respective lysine nitrogen contents (Van Slyke and Birchard, 1914)

Both the globulins compare favourably with casein and contain requisite amounts of arginine, histidine and lysine The arginine content of both the globulins is higher than that of casein, being nearly double in the case of the globulin of *Pisum arvense* (*vide* Narayana and Sreenivasaya, 1928) The two globulins are, however, very deficient in both cystine and tryptophane These legumes are usually taken with cereals and are useful in supplying the essential diamino acids especially lysine in which the cereal proteins are usually deficient

From the metabolism data (Tables VI and VII), the average biological values at a 5 per cent level of intake, for casein and the globulins of *Cajanus indicus* and *Pisum arvense*, have been found to be 89, 69 and 48 respectively At the same level of intake, the biological values for the whole grains of the two pulses are slightly higher, namely, 79 and 69 as against 69 and 48 for the corresponding globulins These results clearly show that there is a supplementary relationship between the different proteins existing in the seed and that they make good, to a certain extent, each others deficiencies as regards their food value As a certain fraction of the nitrogen content of each pulse grain exists in a non-protein form, the biological value obtained in such a case refers to the total nitrogen of the grain and not to its total protein The level of intake of protein, viz , 5 per cent, seems lower than the minimum required for maintenance Feeding experiments at a higher level of protein intake are still in progress

Summary

The globulins, which form the bulk of the proteins in the pulses, *Cajanus indicus* and *Pisum arvense*, have been isolated and analysed by the Van Slyke method Arginine, tyrosine, tryptophane and cystine have been estimated by individual methods Both the globulins have been found to be deficient in cystine and tryptophane

The biological values for the isolated globulins and also for the whole grains, at an approximately 5 per cent level of protein intake, have been determined

In conclusion we take this opportunity of expressing our gratitude to Dr Jivraj N Mehta, the Dean of our College, for his help and continued interest during the progress of this work We are indebted to the Indian Research Fund Association for defraying the expenses of this investigation

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RAT-FLEA SURVEY OF RANGOON

Part I

THE PORT AREA

PERIOD FROM 5TH JANUARY, 1928 TO 4TH JANUARY, 1929

BY

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PLAGUE was introduced to Rangoon from Calcutta in 1905. Since then and up to the period of this survey, the town has not been free of human cases for a single month. The disease is endemic and the town has been regarded by some as one of the principal foci of infection in the Far East. At no time, however, has the human infection been intense and so far as the facts are known the same applies to the rat epizootic.

Early attempts to determine the rat-flea distribution in Rangoon town were made in 1919 and 1921 by Cragg, and later in 1923 and 1924 by Jolly (unpublished). The figures obtained by them are as follows —

Year	Period of year	Percentage of *cheopis*	Percentage of *astia*	Number of fleas collected
1919	December	45·6	54·4	181
	July	60·0	40·0	
1921		57·5	42·5	221
1923	May–December	54·2	45·8	1,710
1924	January–May	25·7	74·3	412

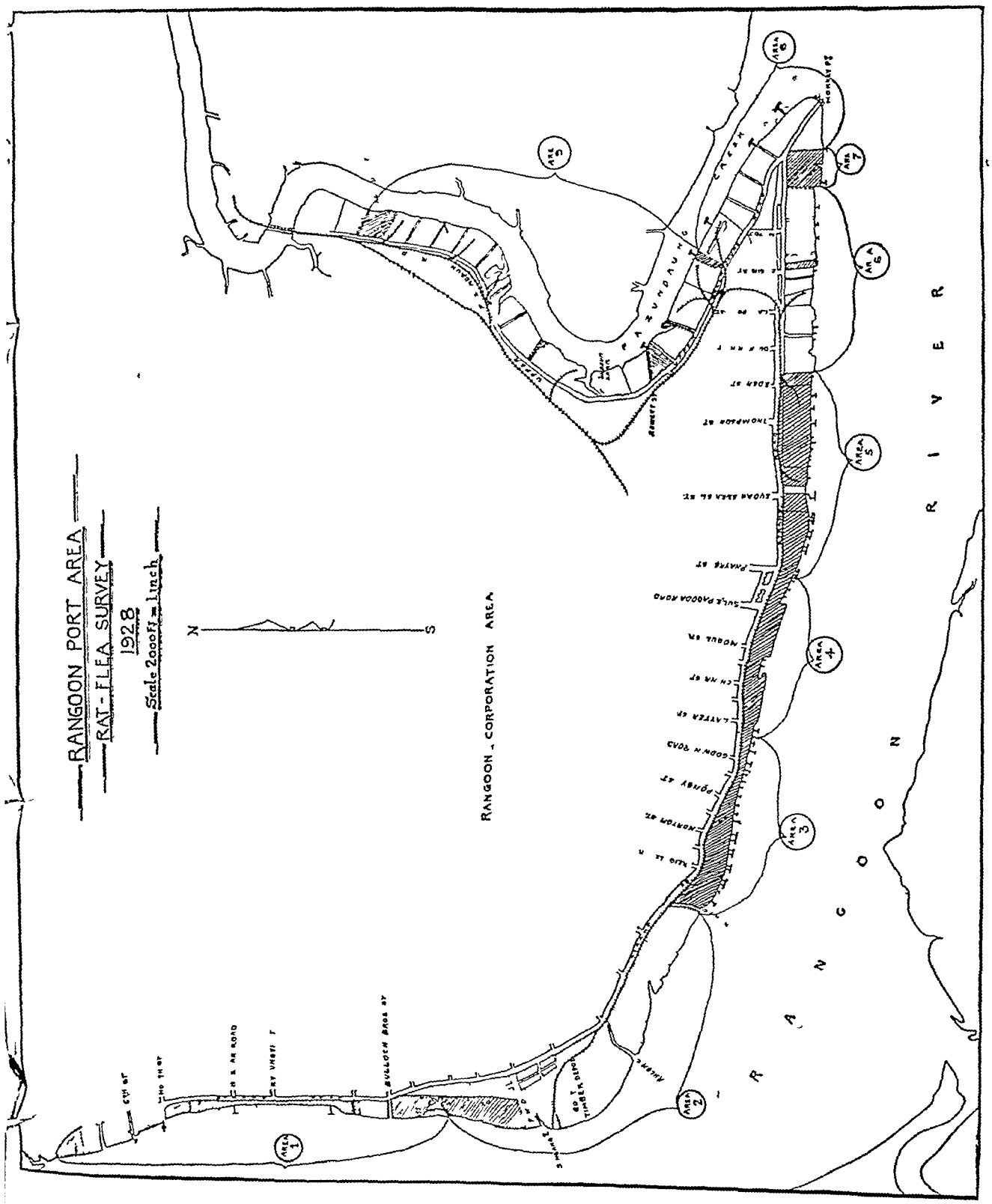
The numbers of fleas collected are small, but the discrepancy between those obtained in 1919, 1921 and 1923, and those recorded in 1924 is striking In the period 1919–1923 the proportion of *X cheopis* ranged from a lowest of 45 6 per cent in December 1919 to a highest of 60 per cent in July of the same year, the figures for 1921 and 1923 being over 50 per cent, while in 1924 the figure had dropped to 25 7

The present survey was originated by one of us (G G J) in 1928, with the object of investigating more fully the rat-flea distribution, in the hope of adding to our knowledge of plague conditions in Rangoon and of the rôle of this seaport town as a centre in the dissemination of plague by land and sea

It was decided in the first instance to begin the rat-flea survey in the Port Area This is a strip of land under the administration of the Rangoon Port Trust, bordering the Rangoon River on the west, south and east of the town, about 12 miles long and having an average depth of approximately 100 yards The Port Health Officer, Captain C G Crow, KIH, IMD, is in medical and sanitary charge of this area, and we are indebted to him for placing his ratting gangs at our disposal and for assisting us in other ways

We divided the Rangoon Port Area (*vide* Map) into nine sections numbered 1 to 9, and surveyed all the Port Commissioners' premises in these areas Rats were trapped for the most part indoors, but the localities varied from old mud-floored grain warehouses built of timber, to modern well-constructed concrete and galvanized iron ones, from mat and thatch native huts to well-built masonry houses, and from roadside refreshment stalls to timber stacks At the outset our staff was too small to deal with all areas simultaneously, and it was decided to concentrate upon area 4, which embraces the Latter Street and Sule Pagoda wharves, and includes the main berthing accommodation for sea-going vessels with the necessary transit sheds and warehouses for Burma's imports Rice exports are mainly shipped in the stream, direct from barges, which are loaded from individual mills, some of them in Rangoon, while others are situated up-country, the barges in the latter case bringing the rice to Rangoon down the Irrawaddy River or from various points in the Delta From July to September there is a considerable coastal export trade in potatoes from Latter Street wharf Area 4 has a water frontage of nearly a mile

At first it was only possible to deal with area 4, and from this area ten rats a day were examined Later in the survey, it was considered desirable to extend the field of operations, and the financial assistance of the Indian Research Fund Association was sought This body generously came to our assistance and provided additional funds, by which it became possible to engage extra staff, and to collect a total of 40 rats a day from five additional areas, viz, areas 1, 3, 5, 7 and 9 Areas 2, 6 and 8 were not surveyed as the staff was insufficient, and these areas were not considered of such great importance as the others, area 2 being a timber area, area 6 timber and oil, and area 8 mainly timber Ten rats a day continued to be examined from area 4


RANGOON PORT AREA
RAT-FLEA SURVEY
1928
Scale 2000ft = 1 inch
N
S
RANGOON - CORPORATION AREA
RANGOON RIVER
AREA 1
AREA 2
AREA 3
AREA 4
AREA 5
AREA 6
AREA 7
AREA 8
AREA 9
PANSODAN CREEK
BULLOCH BROS ST
PUNJAB ST
NORTON ST
PUNJAB ST
GODWIN ROAD
CANTEEN ST
CHINA ST
NORAH ST
SULE PAGODA ROAD
PANTRA ST
SUDAN ARAB ALLEY
THOMPSON ST
ADAM ST
GUN ST
DOCK ST
TIMBER DUMP

throughout the year, and, with effect from the 5th May, 6 rats a day were collected and examined from each of the other areas numbered 1, 3, 5, 7 and 9

A brief description of the premises surveyed in each of these other areas is called for

Area 1 contains on the south timber yards and the residential houses of the dealers and their employees, these houses mainly double-storied Next we skip a rice mill and come to warehouses holding rice and tobacco, the rice sheds being next door to the mill, and the ground around them heavily honeycombed with rat-holes Next is a strip of foreshore with wood and brick houses Beyond is a timber and rice mill area, extending up to the Hanthawaddy Road, which is bordered by a row of timber houses, and an old mud-floor rice godown where many rats were caught

Area 3 includes the Crisp Street rice market to which rice is brought from the small mills bordering the river in the vicinity of Rangoon, sold and distributed by boat throughout the delta of the Irrawaddy River Next are warehouses storing cocoanuts, onions, dhal, potatoes and wheatflour, mostly imported, then sold, reloaded in small boats and taken away by water for distribution in the delta Next are the jetties of the Irrawaddy Flotilla Company where the warehouses take miscellaneous goods for inland water transport Other warehouses in this area hold rice for export to Malabar, Ceylon, Calcutta, Malaya, Java, etc The main rice traffic through the Port Commissioners' sheds is from Area 3, the rice being chiefly loaded into lighters and shipped in the stream The area also includes a fish market, and watchmen's quarters in the upper storey of an old timber warehouse

Area 5 consists mainly of modern well-built concrete-floored transit sheds, holding goods for river transport between Rangoon, Mandalay and intermediate ports They also store beans, rice, onions, groundnuts, tobacco, cutch, sesamum, maize, edible oils, etc , for export to foreign countries, these goods being loaded in the stream from barges The area further includes the Arakan and Tenasserim Coastal Depôts To the west are the Port Commissioners' workshops, an area from which launches ply to certain riverine ports, the Port Health Station, where over 300,000 coolies enter and leave Burma each year, a block of menials' quarters, and a rest-house for deck passengers

Area 7 is a small compact area, consisting mainly of residential quarters for the Port Commissioners' staff, and includes well-built modern brick bungalows for officers and wooden shingle-roofed quarters for subordinates It also includes mineral oil godowns and menials' quarters

Area 9 includes a large salt depôt, warehouses storing bran, tobacco, etc, a police station, coal depôt with labourers' quarters, and streets of masonry lodging-houses for coolies and the wooden houses of Burmese of the 'clerk' class

Rat collection—The method of collection of rats was as follows Traps of a simple cage pattern with snap door were used These were baited with bread, and were set each evening in the several areas by members of the port

ratting gang Each area was allotted a distinctive colour and the traps used were marked to correspond The traps were set each day in a different part of the area, the site being selected by an overseer under our general supervision More traps were used than were needed to produce the necessary catch of rats for examination in order to ensure that the number caught would not fall short of our requirements In the early morning the traps were collected, and to each trap containing a rat a label was affixed The traps from each area were then collected together at a central point, and from them a random selection of the number necessary to produce the required quota from that area was made by the gang overseer in charge The selected traps each containing one rat were then placed within stout calico bags to prevent loss of fleas, and conveyed by hand cart to the Harcourt Butler Institute of Public Health

Flea collection—At the Institute the traps, still in their bags, were collected and half a fluid ounce of petrol was poured on each bag The traps were then placed in a large air-tight wooden box and left there for 20 minutes, by which time rats and fleas had been killed The traps were next taken from the box, removed from their bags one by one, and placed on a white sheet Each rat was tumbled out on to the sheet and the trap well tapped to shake out any fleas loose in it The rat was then combed carefully for fleas and the latter collected In a few rare instances in which more than one rat was found within a trap the fleas obtained were allocated equally between the rats Each rat was identified and its particulars entered upon the label

The catch of fleas from each rat was placed in a separate Wright's agglutination tube, which was marked with the colour of the area from which the rat had been collected, and with the serial number of the label on the trap

The fleas were then treated with liquid carbolic acid, sufficient acid being put in each tube to cover the fleas, and the tubes were placed in a wire rack, resting in a tin basin containing paraffin wax over a sand bath, and the whole raised to 120°C, and thereafter allowed to cool to room temperature The fleas from each tube were next cleared in xylol and mounted in canada balsam in a row or series of rows on a glass slide The cleared specimens were identified under a 2/3rd objective, the preliminary treatment having rendered them so pellucid that the characteristic identification points could be picked out at a glance The identification of all fleas was carried out by one of us (V W F) After identification the flea particulars were entered on the corresponding label

Altogether in the period under review 7,293 rats of seven different species were collected Table I shows in summary form the results obtained from the survey

Average number of rats per trap—This is shown in Table II The highest number of 36 1 rats per 100 traps was obtained in February 1928, and on the whole there is a progressive diminution in rats caught per 100 traps from month to month, the drop from April to May being pronounced, while during the last four months October 1928 to January 1929, the figures are fairly steady It

TABLE I

Total number of working days	252	
Total number of rats examined	7,293	
Total number of fleas obtained	18,884	
Total number of *X astia*	17,891	94 76 per cent
Total number of *X cheopis*	990	5 24 „
Average number of fleas per rat	2 59	
Average number of *X astia* per rat	2 45	
Average number of *X cheopis* per rat	0 14	

was in the month of May that trapping on a more extensive scale began, and this may account for the drop in number of rats per trap from April to May Probably towards the end of the period conditions had become stabilized and the number of rats per trap therefore approached constancy

Rat species by months—Table II gives the rats caught by months and by species It is remarkable that the two species *Mus concolor* and *Nesokia bengalensis* together formed 62 per cent of the total rats caught These two rats have very different habits of life, the former being the indigenous house rat of Burma, while the latter is a species which lives in burrows outside houses *Mus concolor* and *Nesokia bengalensis* must be regarded as the two common rats of the Port Area It is important to note that *R rattus* which is of such outstanding importance in other parts of India is of much less importance in Rangoon where its place is taken by *M concolor* Table II shows that *R rattus* formed only 8 83 per cent of the total rats caught whereas *M concolor* formed 31 42 per cent

Distribution of rats by areas—Table III gives this and it will be observed that the species distribution is substantially uniform throughout all the areas examined

Sex distribution of rats—An interesting feature is that out of a total of 7,293 rats caught only 1,644 were males, the females caught being 77 46 per cent of the total The sex disparity was most marked in the case of *R norvegicus* with a rate of over 12 females to each male caught, and least marked in the case of *Mus concolor* where the rate was 2 females to each male

Rat flea indices—The average number of fleas per rat was 2 59 The figure–*vide* Table V–varied from the very high index of 11 76 in February to 0 50 in September The flea index fell abruptly in the month of June, by which time the rains were well established, kept dropping to September, then rose again to over 3 by January 1929, the four months of July, August, September and October having indices of below 1

The *X astia* index shows the same features as the total flea index, owing to the overwhelming preponderance of this flea throughout the year, the figures being *X astia* 94 76 per cent and *X cheopis* 5 24 per cent (*vide* Table I) The remarkably high flea indices of February and March are therefore due to

TABLE II

Distribution of rats by months

Months	Average number of rats caught per 100 traps set	Total rats caught	Species of rats					
			M concolor	*N bengalensis*	*M musculus*	*R norvegicus*	*R rattus*	*C cœrulea*
January 1928	26·26	173	37	68	7	45	10	6
February	36·10	180	37	75	22	31	14	1
March	27·28	209	62	87	19	14	13	14
April	27 22	195	23	58	53	33	18	10
May	18·33	687	106	332	66	114	19	50
June	17 32	650	163	202	69	119	69	28
July	16·95	879	241	327	151	32	100	28
August	17·06	920	315	223	172	84	91	35
September	16·99	800	311	129	154	110	76	20
October	14 92	864	288	214	110	138	89	25
November	13 87	776	341	214	80	83	53	5
December	14·79	840	333	259	64	59	81	44
January 1929	14 89	120	34	46	9	9	11	11
TOTAL			2,291	2,234	976	875	644	277
PERCENTAGE			31 42	30·63	13·38	11·94	8·83	3·80

TABLE III

Percentage distribution of rats per area

Species	Area No 1	Area No 3	Area No 4	Area No 5	Area No 7	Area No 9	All areas
Mus concolor	36 15	34 09	27·25	34·41	29·90	33·13	31 42
Nesokia bengalensis	27 58	28·55	34 42	28·97	28·09	30·15	30·63
Mus musculus	12·23	13·38	14 04	11 13	14 85	13·68	13·38
Rattus norvegicus	12·13	11 02	12·14	11 85	13·26	11·00	11 91
Rattus rattus	9 19	9·26	6 82	10·52	10·82	9·56	8·83
Crocidura cærulea	2·72	3·70	5 33	3 09	3·08	2·18	3 80

TABLE IV

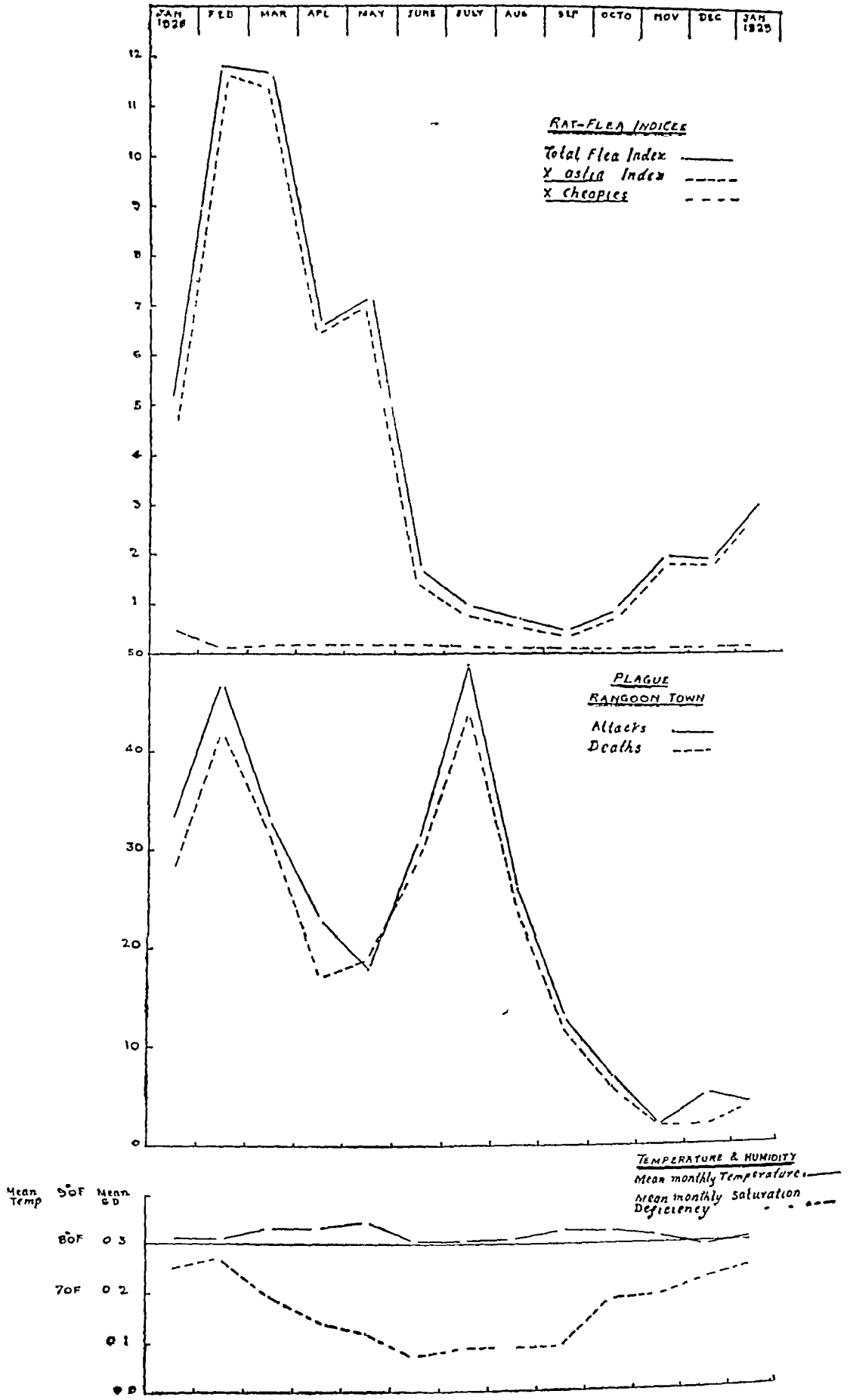
Sex distribution of rats by species

Species	Males		Females		Total both sexes
	Total	Sex Per cent	Total	Sex Per cent	
Mus concolor	762	33·26	1,529	66·74	2 291
Nesokia bengalensis	448	20·05	1,786	79·95	2,234
Mus musculus	162	16 59	814	83·41	976
Rattus norvegicus	71	8·15	800	91 85	871
Rattus rattus	127	19·72	517	80·28	644
Crocidura cærulea	74	26 71	203	73·29	277
TOTAL	1,644		5,649		7 293

TABLE V

Distribution of fleas by months

Months	Number of working days	Total number of rats examined	Total number of fleas obtained	Total number of *X astia* obtained	Total number of *X cheopis* obtained	Average number of fleas per rat	Average number of *X astia* per rat	Average number of *X. cheopis* per rat
January 1928	19	173	878	792–(90 35)	86–(9 65)	5 08	4 58	0·50
February	18	180	2,117	2,093–(98 86)	24–(1 14)	11 76	11 63	0·13
March	21	209	2,432	2,386–(98 11)	46–(1 89)	11 64	11 42	0·22
April	20	195	1,290	1,250–(96·90)	40–(3·10)	6·62	6·41	0·21
May	21	687	4,937	4,810–(97 43)	127–(2·57)	7 18	7·00	0·18
June	20	650	1,090	956–(87·70)	134–(12·30)	1·68	1 47	0·21
July	22	879	851	709–(83·31)	142–(16 69)	0·97	0·81	0·16
August	23	920	656	543–(82 77)	113–(17 23)	0·71	0·59	0·12
September	20	800	402	340–(84 58)	62–(15 42)	0·50	0·42	0·08
October	23	864	754	689–(91 38)	65–(8·62)	0·88	0 80	0·08
November	21	776	1,520	1,454–(95 66)	66–(4 34)	1 95	1 87	0·08
December	21	840	1,595	1,527–(95 74)	68–(4·26)	1 90	1 82	0·08
January 1929	3	120	362	345–(95·31)	17– (4 69)	3·02	2·88	0·14


JAN 1928
FEB
MAR
APL
MAY
JUNE
JULY
AUG
SEP
OCTO
NOV
DEC
JAN 1929
RAT-FLEA INDICES
Total Flea Index
X Oriental Index
X Cheopis
PLAGUE
RANGOON TOWN
Attacks
Deaths
TEMPERATURE & HUMIDITY
Mean monthly Temperature
Mean monthly Saturation Deficiency
Mean Temp
Mean SD
80F
70F
0 3
0 2
0 1
0 0

X astia, and the great seasonal variation in the flea index is almost entirely due to fluctuation in the numbers of *X astia*, the number of fleas found in February and March being 23 times the number in September

When we come to *X cheopis* we find the indices less variable Leaving out for the moment the figure of 0 5 for January 1928, the monthly *X cheopis* index only varied between a highest of 0 22 in March and a lowest of 0 08 in the months of September, October, November and December The *X cheopis* index of 0 5 in January 1928 followed upon a low mean temperature of 77 1°F recorded for December 1927, which was the coolest month in Rangoon in the three years period 1926, 1927 and 1928 As pointed out by Hirst (1926) the range of temperature between 70°F and 80°F is favourable to *X cheopis* reproduction, provided that the drying power of the air is not greater than that represented by a vapour pressure deficiency of 0 3 inch of mercury The vapour pressure deficiency in Rangoon in December 1927 was 0 177, so that the month was an exceptionally favourable one for *X cheopis* reproduction The most striking feature of Table V (illustrated better in the Graph —*see* opposite) is the remarkably low *X cheopis* index, to which further reference will be made

The high total flea index obtained in the months of February and March 1928 suggested further investigation, and therefore Table VI was prepared, which demonstrates that the great flea increase in these months was not confined to any one species of rat or to the outdoor rats as compared with the indoor or vice versa, but the proportionate increase is greater in certain species than in others, and is particularly marked in the case of *R rattus* This rat formed 8 83 per cent of the total rats trapped and, as the numbers were small in the months under consideration, the probable error involved is correspondingly great We are only justified therefore in noting that the marked increase in the rat-flea index in February and March was exhibited by all species of rat and was therefore due to some cause common to all species

Distribution of fleas by areas—This distribution given in Table VII below, is surprisingly regular, and shows that, whatever may be the fleas distribution in other parts of Rangoon, we have a homogeneous area in the Port

Distribution of fleas by species of rats—Table VIII shows the distribution of the fleas found on the various rats *Crocidura cærulea* has been included in this and other tables because, although there is no reason to believe that it plays any part in the spread of plague, it harbours both *X cheopis* and *X astia* and was frequently met with and trapped during the survey

Table VIII illustrates two interesting points The first is the close correlation between the weight of the rat and the total number of fleas found Column 2 of the table gives the average weight of 100 consecutive fully grown adults of each species It will be noted that generally the number of fleas per rat corresponds with the average weight Excluding *Crocidura cærulea* whose habits of life are rather different from the true rats, the only discrepancy is in the figures for *Nesokia bengalensis* and *Rattus norvegicus* This discrepancy

TABLE VI

Average number of fleas per rat by species and months

Species of rat	Jan 1928	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan 1929
Mus concolor	1 92	3 67	2·35	1 13	1 54	0 61	0 51	0·32	0·26	0·31	0 37	0·41	1 03
Mus musculus	0 43	0·68	1 47	0 64	1 00	0 19	0 28	0 12	0 07	0 15	0·18	0·25	0 22
Rattus rattus	0 50	7 14	4 62	1 11	0·81	0 84	1 15	1 09	0 81	0·92	2 13	1 47	1·91
Nesokia bengalensis	6 25	16 77	20 61	14 72	15 83	2 16	1 53	1 16	1 13	1 12	1 57	3·53	5 13
Rattus norvegicus	6 24	20 61	28·29	10 51	12 89	3 17	1·47	1 94	0·90	1 75	3·20	6 50	7 22
Crocidura cærulea	0·17	1 00	0·64	1 80	1 33	0·29	0 50	0·31	0 25	0·10		0·36	0 27

TABLE VII

Percentage distribution of rats per area

Species	Area 1	Area 3	Area 4	Area 5	Area 7	Area 9	All areas
X cheopis	7 92	5 99	3 77	6 07	7·52	6·75	5 24
X astia	92·08	94 01	96·23	93·93	92 48	93·25	94 76

TABLE VIII

Distribution of fleas by species of rat

Species of rat	Average weight of adult specimen in ounce	Fleas per rat	*X astia* per rat	*X cheopis* per rat	Number of *X cheopis* per 100 *X astia*
Nesokia bengalensis	12·31	1 59	1 47	0 1083	2·00
Rattus norvegicus	9·87	4 99	4 7815	0 2071	4·33
Rattus rattus	3 50	1 60	1 2783	0 3117	24 38
Crocidura cærulea	1·65	0 53	0 3686	0 2608	7 07
Mus concolor	1 12	0·56	0 4530	0 1098	24 23
Mus musculus	0 52	0 28	0 2217	0 0571	25 75

is probably due to the correct correlation being between the available skin area rather than the weight of the animal, *Rattus norvegicus* having a greater skin area in proportion to its weight than *Nesokia bengalensis* which is a burly thick-set rat

The second point of interest is the marked preference shown by *X cheopis* for the three rats *Mus concolor*, *Rattus rattus* and *Mus musculus* These rats all show a *X cheopis* percentage of approximately 25, compared with 4 33 for *Rattus norvegicus* and 2 00 for *Nesokia bengalensis* *Crocidura cœrulea* on the other hand gives a figure of 7 07 *Mus concolor*, *Rattus rattus* and *Mus musculus* are all house rats, while *Rattus norvegicus* and *Nesokia bengalensis* live outside houses and *Crocidura cœrulea* is betwixt and between It appears reasonable therefore to associate the preference of *X cheopis* for the house rats with the more equable conditions of temperature and humidity which prevail indoors However this may be, the relatively high *X cheopis* figure on these rats is in keeping with the recognized greater danger of human plague from these domestic species than from the outdoor ones The main rôle of the outdoor rats in the spread of human plague is to pass the epizootic to the house rats from which the human infection is derived, and it is an interesting point that *X cheopis* should exhibit such a marked preference for the domestic species

Sex distribution of fleas—Bacot, Petrie and Todd in Egypt (quoted by Hirst, 1926) and Hirst (1926) in Colombo have made observations on the proportion between the sexes of rat-fleas, with somewhat varying results Table IX gives the figures we have obtained which show a preponderance of females of both species particularly in the case of *X astia*

TABLE IX

Sex distribution of fleas

Species	Males		Females	
	Total	Sex Per cent	Total	Sex Per cent
X cheopis	447	45 15	543	54 85
X astia	6,721	37 56	11,173	62·44

SUMMARY

1 The Rangoon Port Area was surveyed over a period of twelve months and 7 293 rats examined, producing 18,884 fleas

2 The distribution of the various species of rats and fleas throughout the various section of the area surveyed was markedly homogeneous

3. *M. concolor* and *N bengalensis* were the commonest rats found, being present in nearly equal proportions, and together forming 62 per cent of the rats examined *R rattus* formed only 8 83 per cent of the total

4. The indoor rats comprising *M concolor*, *R rattus* and *M musculus* formed 53 63 per cent and the outdoor rats, namely *N bengalensis* and *R norvegicus* 42 57 per cent, while *C cœrulea* which is of ambiguous habits formed 3 80 per cent

5 The average number of rats per 100 traps varied from 36 10 in February to 13 87 in November

6 For all species of rats the flea index was 2 59, the *X astia* index being 2 45 and the *X cheopis* index 0 14

7 There was a pronounced seasonal fluctuation in the number of *X astia* while *X cheopis* remained at a relatively low level throughout

8 The survey, while demonstrating a low prevalence of *X cheopis*, brings to light a marked relative preference of this flea for the indoor rats as opposed to the outdoor rats

9 Tables are given indicating a relationship between the number of fleas found per rat, and the weight of the rat, and showing the sex distribution of rats and fleas by species

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ESTIMATION OF STATURE FROM LONG BONES IN INDIANS OF THE UNITED PROVINCES A MEDICO-LEGAL INQUIRY IN ANTHROPOMETRY

BY

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THIS investigation was undertaken on the suggestion of the head of the Department of Forensic Medicine who informs me that there are no authentic data for the population of these provinces The question he told me very often comes up in judicial proceedings but for want of any authoritative figures has to be given up altogether as a help in identification He is of opinion that with the figures available in the textbooks (derived of course from observations on European material) the margin of error in estimating the stature from a long bone is 8–10 inches The British authorities are of a similar opinion even about British figures as the following few quotations show —

Glaister says, 'We are disposed to discount very largely any conclusions from averages Any conclusion so arrived must depend for its correctness upon the presence of half of the skeleton , otherwise it cannot be considered free from error to the extent probably of several inches' *Aitchison Robertson* says, 'From a single bone it is almost impossible to determine the stature with any degree of exactitude' *Dixon Mann* says, 'Estimation of stature from the measurements yielded by one or two bones are very unreliable , they partake largely of the nature of guess work' Devergee is of opinion that these estimations are liable to lead to an error of 5 inches at least

It is well known that the proportions vary in different races and so the application of figures, derived from European material for estimation of stature from long bones in these provinces, would in any case be open to serious objection, and when these same figures even for Europeans are considered by

the authorities as of doubtful value, the need for an investigation of this nature becomes very obvious

Apart from the applicability of results derived from one race to another the chief source of error in the various observations so far made appears to me to have been, that the average proportion has been arrived at merely by calculating the average stature of say a hundred bodies and the average length of a certain long bone calculated from measurements of one hundred such bones, not necessarily belonging to the same bodies, from whom the average stature was calculated It is obvious that the result will be more accurate if the measurements are taken on bones of the same body whose stature was first measured and the averages then worked out

My observations discussed later in this paper have been made on material so collected that the bones measured could be identified as belonging to the bodies whose stature was first measured, and so this source of error in estimating averages was eliminated These measurements have all been made on male adults only as it was realized that introduction of female and immature bones will vitiate the results Further as there is usually some difference between the lengths of bones of the two sides the length of a bone from a body was taken to be the mean, between the lengths of bones of the two sides These measurements on macerated bones were of course all on bones without the articular cartilages and so a millimetre on either end will have to be allowed for in the practical application of these results All bones with any suspicion of disease were rejected for the purpose of these measurements

The instruments used in these measurements have been the Osteometric Board, Slide Compass and Flower's Craniometre used as Callipers

The total number of bones examined was 100 in the case of the bones of the upper extremity and 80 in the case of the lower extremity These observations are appended in the tables at the end of this paper

Humerus

This bone was measured by means of the Osteometric Board—the lower edge of the trochlea touching one end and the highest point on the head of the bone the other The average of this measurement works out to 306 mm and as the average stature is 1,636 mm the length of a given humerus would have to be multiplied by $\frac{1\,636}{306} = 5\,3$ to get the stature I propose calling the figure thus arrived at 'Multiplication Factor' or M F in the course of this paper Therefore one can summarize by noting that M F of the humerus is 5 3 (*vide* Table I)

Radius

This bone was measured by means of an Osteometric Board from the highest point of the margin of the head to the point of the styloid process M F of Radius works out to 6 9 (*vide* Table I)

Ulna

Ulna was measured by means of an Osteometric Board from the tip of the olecranon process to the tip of the styloid process M F of ulna works out to 6 3 (*vide* Table I)

TABLE I

Serial No	Stature in mm	Length in millimetres		
		Humerus	Ulna	Radius
1	1,505	274	223	204
2	1,508	277	224	205
3	1,530	279	231	214
4	1,532	280	234	215
5	1,540	297	252	232
6	1,540	297	254	232
7	1,546	285	243	222
8	1,515	292	248	227
9	1,574	305	260	242
10	1,577	307	256	243
11	1,578	292	259	238
12	1,581	297	258	237
13	1,584	303	252	229
14	1,586	307	257	240
15	1,593	302	253	231
16	1,596	306	261	240
17	1,599	305	250	227
18	1,599	311	262	240
19	1,631	296	246	229
20	1,635	302	268	245
21	1,636	300	270	248
22	1,645	299	256	241
23	1 647	306	260	244
24	1,654	295	250	230
25	1,655	298	262	242

TABLE I—*concld*

Serial No	Stature in mm	LENGTH IN MILLIMETRES		
		Humerus	Ulna	Radius
26	1,656	297	254	236
27	1,659	299	243	227
28	1,659	299	263	243
29	1,663	302	246	230
30	1,663	312	267	240
31	1,669	310	256	236
32	1,669	315	269	244
33	1,672	304	252	233
34	1,674	312	269	246
35	1,675	318	271	249
36	1,677	316	265	242
37	1,679	314	263	242
38	1,680	298	270	255
39	1,685	316	264	243
40	1,685	324	270	251
41	1,687	322	255	236
42	1,692	321	254	234
43	1,692	325	273	251
44	1,702	317	268	244
45	1,705	330	280	262
46	1,720	322	266	244
47	1,720	332	278	262
48	1,724	327	266	243
49	1,735	331	274	257
50	1,739	334	276	255

Femur

This bone was measured by means of an Osteometric Board by first placing the two condyles in contact with the fixed end, the shaft thus lying obliquely,

and then shutting down the movable piece on the head M F for femur works out to 3 7 (*vide* Table II)

Tibia

The measurement taken on this bone was the one known as the condylo-malleolar length from the proximal articular surface of the internal condyle to the tip of the malleolus M F for the tibia works out to 4 48 (*vide* Table II)

TABLE II

Serial No	Stature in mm	Length in millimetres		
		Femur	Tibia	Fibula
1	1,477	389	317	309
2	1,487	392	317	316
3	1,495	396	334	330
4	1,504	407	331	337
5	1,505	403	337	335
6	1,505	410	330	340
7	1,506	405	338	337
8	1,507	405	341	343
9	1,511	419	352	354
10	1,512	408	343	346
11	1,515	411	347	342
12	1,518	416	344	338
13	1,547	431	358	358
14	1,572	437	362	357
15	1,575	431	335	356
16	1,583	432	366	364
17	1,595	437	365	362
18	1,596	439	364	359
19	1,613	430	357	355
20	1,619	435	358	353
21	1,620	436	359	357
22	1,624	442	371	365

TABLE II—*contd*

Serial No	Stature in mm	LENGTH IN MILLIMETRES		
		Femur	Tibia	Fibula
23	1 627	429	369	365
24	1 630	434	366	363
25	1,636	449	368	370
26	1,640	444	371	373
27	1 652	442	373	366
28	1 675	447	366	367
29	1,680	452	366	369
30	1,681	450	375	372
31	1 682	451	381	384
32	1 682	458	383	383
33	1 684	455	371	368
34	1,690	460	371	364
35	1 691	447	385	383
36	1 691	452	373	373
37	1,697	451	387	383
38	1,737	470	381	377
39	1,740	472	382	379
40	1,747	471	385	385

Fibula

The measurements of this bone are not so useful for our purpose for this bone is often found with the ends worn off If intact the maximum length possible with the Osteometric Board is taken M. F for this length of fibula works out to 4 48 (*vide* Table II)

The measurements above selected in each bone are not the best for practical purposes as the ends of many bones like the styloid processes may be absent in medico-legal material or, as in the case of the femur, the length measured as above will vary according to the angle of the neck which as is well known varies at different life periods I am therefore calculating the M F for other more preferable measurements in some of these bones These results will be published as a separate note later on

In order to test my results and to estimate the amount of error one is liable to make in the estimation of stature from a single long bone according

to the above figures, I prepared another series of 15 upper extremity and 12 lower extremity bones where the stature of the body from which these bones were derived was known The error in the estimated stature as worked out according to my results is shown in millimetres in the Tables A and B for the bones of the upper and lower extremities respectively

The average error in estimating the stature from a single bone in this series in Table A works out to 27 mm , 40 mm , and 35 mm in the case of humerus, radius and ulna respectively Putting it differently it can therefore be stated that it is possible to estimate the stature of a body from its humerus within $1\frac{1}{4}$", from its radius within $1\frac{3}{4}$", and from its ulna within $1\frac{1}{2}$" of the correct figure This result is a marked improvement on the present position with regard to this calculation as brought out in the extracts quoted from the medico-legal authorities at the beginning of this paper Taking the average stature of males in these provinces to be 64" and putting the error even at 2" in the case of the upper extremity, the percentage of error works out to only 3 per cent

TABLE A

Upper extremity

Serial No	Real stature	HUMERUS		RADIUS		ULNA	
		Estimated stature	Error	Estimated stature	Error	Estimated stature	Error
1	1,614	1,664	50	1,697	83	1,663	49
2	1,585	1,552	33	1,587	2	1,556	29
3	1,742	1,696	46	1,711	31	1,709	33
4	1,590	1,653	63	1,649	59	1,606	16
5	1,593	1,632	39	1,642	49	1,625	32
6	1,622	1,621	1	1,711	89	1,701	79
7	1,745	1,733	12	1,738	7	1,732	13
8	1,624	1,637	13	1,683	59	1,663	39
9	1,460	1,431	29	1,442	18	1,456	4
10	1,600	1,563	27	1,593	7	1,606	6
11	1,661	1,627	34	1,704	43	1,682	21
12	1,648	1,674	26	1,649	1	1,669	21
13	1,498	1,494	4	1,386	88	1,411	87
14	1,548	1,561	13	1,600	52	1,631	83
15	1 636	1,648	12	1,649	13	1,650	14

TABLE B

Lower extremity

Serial No	Real stature	FEMUR		TIBIA		FIBULA	
		Estimated stature	Error	Estimated stature	Error	Estimated stature	Error
1	1,590	1,576	14	1,617	27	1,568	22
2	1,742	1,739	3	1,664	78	1,662	80
3	1,622	1,605	17	1,666	44	1,576	46
4	1,593	1,550	43	1,568	25	1,545	48
5	1,507	1,517	10	1,523	16	1,518	11
6	1,460	1,413	47	1,456	4	1,406	54
7	1,524	1,542	18	1,541	17	1,527	3
8	1,590	1,568	22	1,581	9	1,554	36
9	1,704	1,665	39	1,697	7	1,657	47
10	1,624	1,642	18	1,657	33	1,603	21
11	1,636	1,613	23	1,599	37	1,590	46
12	1,490	1,457	33	1,447	43	1,442	48

A similar calculation of error based on 12 tests in the case of the bones of the lower extremity (*vide* Table B) brings out that it is possible to estimate the stature of a body from its femur within 24 mm (1″), from its tibia within 28 mm (or a little over an inch) and from its fibula within 37 mm ($1\frac{1}{2}$″) of the correct figure The percentage of error therefore in the case of the bones of the lower extremity works out to 2 3 per cent

SUMMARY OF RESULTS

(1) The results are expressed in the form of a figure which I have called the M F (Multiplication Factor) for a particular bone The length of a given bone is to be multiplied by its M F to estimate the stature The M F of the various bones according to these observations are as follows —

	M F
Humerus	5 3
Radius	6 9
Ulna	6 3
Femur	3 7
Tibia	4 48
Fibula ..	4 48

It is to be noted that the 'length of a bone' for this calculation means the length as measured between the anatomical points given in the text, when the articular cartilage is absent

(2) Generally speaking it is possible with these figures to estimate the stature of a body from a long bone to within 2″ (although it is less in the case of the bones of the lower extremity)—a marked improvement on the opinions expressed by various authorities on the matter

(3) The figures obtained are primarily for application to the population of these provinces and give valuable data of medico-legal importance

(4) The margin of error, even at the highest, is not more than 3 per cent

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THE EPIDEMIOLOGY OF CHOLERA, WITH SPECIAL REFERENCE TO TRANSMISSION A PRELIMINARY REPORT

BY

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IV LABORATORY INVESTIGATIONS
V CONCLUSION

I INTRODUCTORY

THAT the control of cholera constitutes one of the greatest and most urgent of the public health problems of India few will deny This country is, indeed, the great endemic home of this pestilence and the main source of infection of the rest of the world, and a peculiar responsibility therefore rests upon those responsible for medical policy and practice that every possible effort is made to stamp out this great scourge of the human race But although the contributions made by Indian workers to scientific knowledge of the epidemiology of cholera are both numerous and important, yet it must be admitted that the solution of the problem of the control of this disease is yet to seek It is, however, not generally realized, except perhaps by those with practical experience of cholera epidemics, what little progress has been made in controlling the disease, and it is even less widely recognized that the relative

failure of modern methods of control is not merely the outcome of practical difficulties of an administrative order but is also, in large measure, dependent upon the incomplete state of scientific knowledge of the natural history of the disease

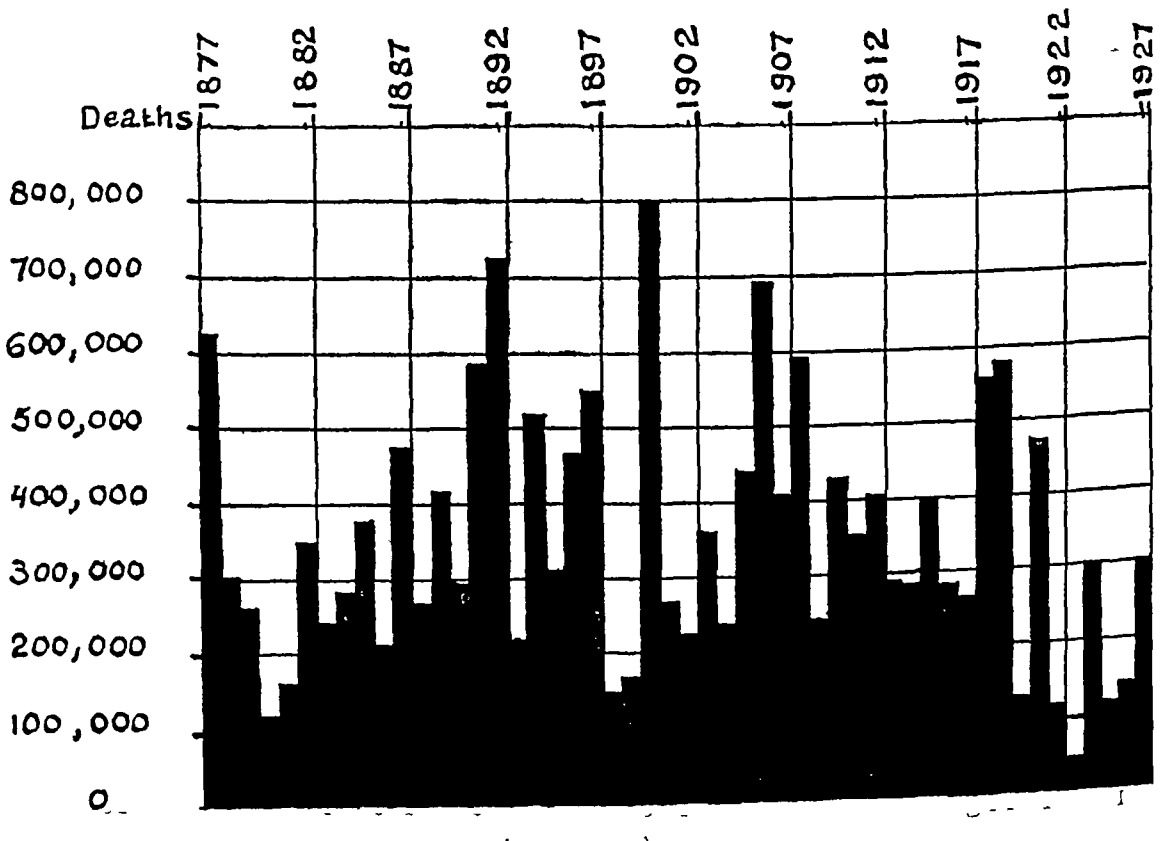
The view, indeed, widely prevails that cholera epidemics can be readily and quickly suppressed by the prompt application of a few simple measures, such as the disinfection of water-supplies, the protection of food and drink, the inoculation of contacts (and pilgrims) and the isolation and treatment of the sick, and it is often assumed that a failure of these measures to control the disease implies lack of energy and resource upon the part of the administrative or executive authorities responsible for applying them

It may, therefore, be well to examine the precise position in respect of the incidence of cholera in India with a view to appraising the progress made during recent years in controlling the disease

The large fluctuations that normally occur in the annual mortality from cholera render it difficult to detect the existence of a trend over a short period of years and it is consequently impossible to determine with precision the effect of preventive measures during recent years in reducing the cholera death-rate It will, however, be seen, from a scrutiny of Chart 1 (which is

CHART 1

Cholera in British India, 1877–1927



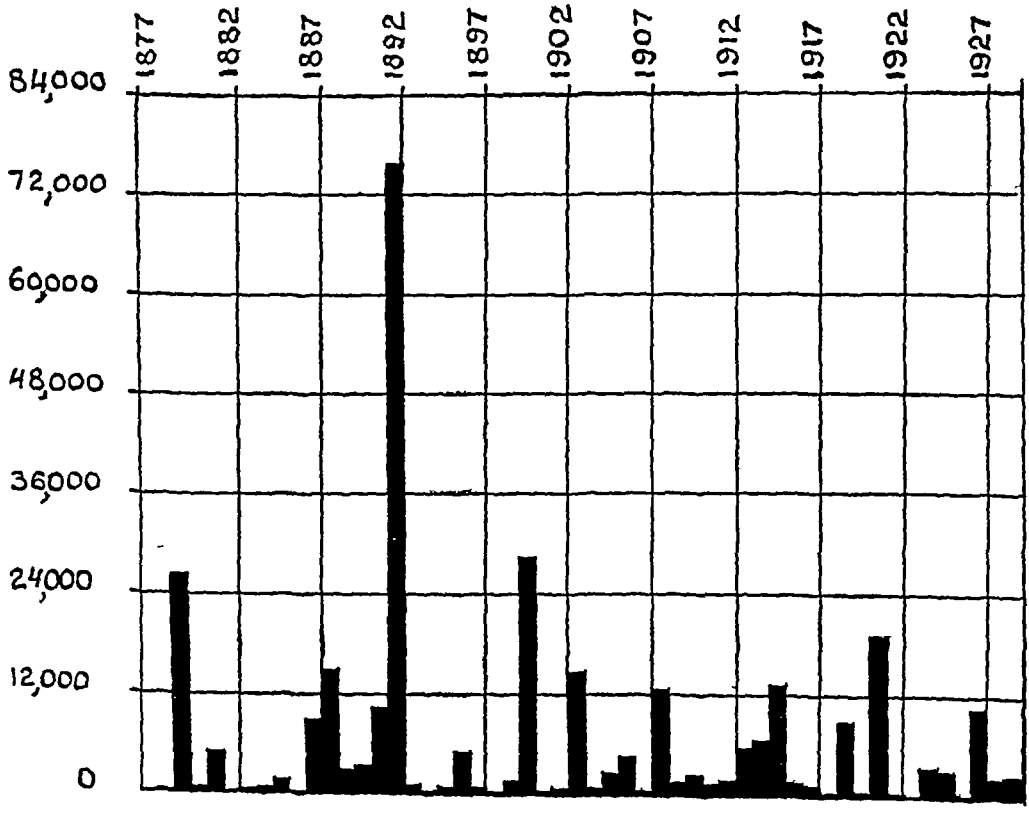
reproduced from the chart published in the *Annual Report of the Public Health Commissioner with the Government of India*, 1927) that no striking reduction in the recorded mortality from cholera in British India and no conspicuous decline in the frequency or intensity of epidemics, is discernible during the period 1877–1927

But if it is not possible to contemplate this chart with any feeling of satisfaction, it is or it may be significant that during the past ten years (1918–1927) the cholera mortality was exceptionally low during five years, whilst during the five years in which the disease was widely prevalent the mortality was relatively low as compared with many previous years of high mortality The same remarks apply to the cholera mortality in the Punjab during this period and a scrutiny of Chart 2, in which the annual cholera mortality during the period 1867–1929 is shown, does not suggest the occurrence of a downward trend during the past ten years

So far as the period 1867–1921 is concerned, a detailed statistical analysis of the mortality figures of the Punjab, carried out by S M Jacob (1923), led this statistician to draw the induction that 'there was no sign of general diminution during the above period'

CHART 2

Cholera mortality in the Punjab, 1877–1929



It is, however, necessary to make allowance for the incomplete nature of the statistical returns during the early part of the above period, and it must also be remembered that improved facilities of transport during recent years may have appreciably increased the opportunities for the spread of the disease and augmented the difficulties of controlling it

It may therefore be that the measure of success attending the efforts made to control cholera during the past ten years has been somewhat greater than the mortality statistics would appear to suggest

Nevertheless, a measure of 'control' which permits of the occurrence of a mortality of approximately 300,000 *per annum* cannot be regarded as satisfactory But even this high figure, which in many years is greatly exceeded, fails to convey any idea of countless tragedies enacted every year by this the most alarming and the most distressing of all epidemic diseases In view therefore of the high incidence of the disease and of the small success that has attended the efforts made to control it, the expediency and indeed the necessity of undertaking a comprehensive study of the epidemiology of cholera is obvious *

The need of carrying out an investigation of this nature came conspicuously to notice when it was found, as the result of observations in the Punjab, that many features exhibited by cholera epidemics could not be satisfactorily explained *in terms of existing knowledge regarding the mode of spread of the* disease It is proposed in this communication to give an account of these observations, but before doing so it should be explained that, on the basis of these observations, the senior author (C A G) reached the tentative conclusion in the year 1927 that it was necessary, in order to account for certain epidemiological features exhibited by cholera, to postulate that some hitherto unsuspected agency was largely concerned in the spread of the disease, and the working hypothesis was eventually formulated that the missing link in the chain of infection was probably the house-fly There is nothing novel attaching to this suggestion, since the house-fly has long been suspected, on evidence which appears to be not entirely conclusive or exhaustive, of acting as a porter of the cholera vibrio, but the postulate it was necessary to make, in order to account for the epidemiological facts, was that the house-fly acted, not merely an accidental mechanical polluter of human food and drink, but as a true biological 'carrier' of the cholera vibrio—in other words that the cholera vibrio undergoes a cycle of development in the tissues of the house-fly and is thereafter disseminated by this insect with the ease and certainty associated with true biological transmission This hypothesis was formulated in November 1928, but as epidemiological observations, like statistical inductions, possess little value until they have been verified and confirmed

* At the instance of the senior author (C A G) the annual conference of research workers held under the auspices of the Indian Research Fund Association at Calcutta in 1928 and in 1929 passed resolutions urging upon the authorities the necessity of undertaking such an investigation

by laboratory experiments, it was necessary to put the hypothesis to the test of crucial experiment This part of the research was conducted by one of us (R B L), but owing to reasons to be detailed later, it was not until August 1930 that the investigation reached a stage when it became permissible to draw tentative conclusions

The results of the experiments, which will be detailed later, are however of so suggestive a nature as to warrant the inference that the house-fly may be a true biological carrier of the cholera vibrio and that this insect may therefore play an important and even essential part in the natural history of cholera

It should be emphasized that the experimental investigation (for which the junior author is solely responsible) is at present admittedly incomplete, but, although these experiments suggest that the cholera vibrio undergoes a cycle of development in the tissues of the house-fly, it is recognized that this conclusion is at present tentative and that it is not, at present, permissible to conclude that cholera must be regarded as essentially an insect-borne disease

II Epidemiological Observations

(*a*) *General Account of Cholera in the Punjab,* 1924–1929

Although the literature of cholera is extremely voluminous, modern researches have been largely concerned with the study of the types and strains of the cholera vibrio and with attempts to determine by means of statistical analyses the part played by climatic and meteorological conditions in the epidemiology of the disease These latter investigations admittedly require to be supplemented by field as well as laboratory investigations, and as this aspect of the subject has not received much attention, no apology is needed for placing on record the result of a study of actual happenings in the field It is proposed therefore in this section to give a general account of the history of cholera in the Punjab during the six years 1924–1929 and thereafter to summarize the salient features of the behaviour of the disease during this period

During the six years 1924–1929, 3,315 outbreaks of cholera have occurred in the Punjab and they were responsible for approximately 40,000 seizures and 22,166 deaths in 298 towns and in 2,917 villages All these local outbreaks, which include also isolated cases, were investigated immediately after their occurrence by officers of the Public Health Department and all important outbreaks formed the subject of detailed investigation In field investigations, it is rarely possible to make observations upon whose accuracy absolute reliance can be placed, nevertheless, it is thought that the facts brought to light in this investigation–though not necessarily the inferences drawn therefrom–may be regarded as substantially accurate These reports, both general and special, have been summarized at the end of each year and it is now proposed to give a brief account of the history of cholera during each of the six years from 1924 to 1929

1924

In 1923 the number of recorded deaths from cholera in the Punjab was eleven—the disease in all instances being imported—but in the year 1924 cholera was responsible for 3,351 deaths (0 16 *per mille*) The source of infection was not, in all instances, traced, but, as in previous years, the first seizures occurred almost exclusively amongst pilgrims returning from the annual spring fair at Hardwar The attendance at Hardwar, in the year 1924, was not unusually large, but it was anticipated that this fair would be the means of spreading cholera in the Punjab (and special preparations were made to meet the threatened danger) because cholera was abnormally prevalent at this time in the province of Bihar and Orissa and in adjoining parts of the United Provinces Cholera began to occur in the Punjab immediately after the conclusion of the fair In many instances the pilgrims developed the disease either during the return journey from Hardwar to their homes in the Punjab or immediately after their arrival, sometimes the pilgrims themselves escaped, whilst the disease attacked their friends and relatives, to whom, according to custom, they had distributed holy water or sweetmeats At this time the disease was confined almost exclusively to Hindus and the infected localities were mainly situated in the districts of the Ambala, Jullundur and Lahore Divisions, which constituted the home of the majority of the pilgrims The number of localities known to have been infected in this manner during April, May and June was 15, but the number of seizures was small, and the total number of deaths up to the end of May was only 24 From the primary foci the disease in most cases disappeared with the death or recovery of the pilgrim, but in a few one or more indigenous cases occurred after an interval of about a week The disease also spread slowly and insidiously to other, usually neighbouring, villages (secondary foci) and the number of outbreaks, as well as the number of seizures in each, steadily increased during the months of July and August The epidemic reached its maximum intensity in the month of September and thereafter rapidly declined until at the end of October the province was completely free During the epidemic, outbreaks occurred in 560 localities, viz, in 56 out of 158 towns and in 504 out of 34,099 villages In most of the 560 infected localities the outbreak was small in extent and short in duration and the mean number of deaths in infected towns and villages was 16 4 and 4 8, respectively In 30 out of the 56 infected towns the deaths from cholera were five or less, in 18 towns, between 6–20 deaths, in four towns, between 21–50 deaths, and in four towns the number of deaths exceeded 50 Towns were relatively severely infected and the urban death-rate was 0 44 *per mille* or more than three times the rural death-rate (0 13 *per mille*)

In no instance did the disease exhibit definite explosive characters and in the four towns in which over 50 deaths occurred, the outbreaks were characterized by the occurrence over a period of several weeks of a small daily number of cases with no apparent connection between them

The salient features of cholera in the year 1924 were therefore the introduction of the disease from Hardwar in April and May and thereafter, after an interval of about 2 months, of the occurrence of large number of small and short-lived outbreaks in towns and villages–and, in the case of a few towns, of outbreaks relatively long in duration and scattered in distribution

1925

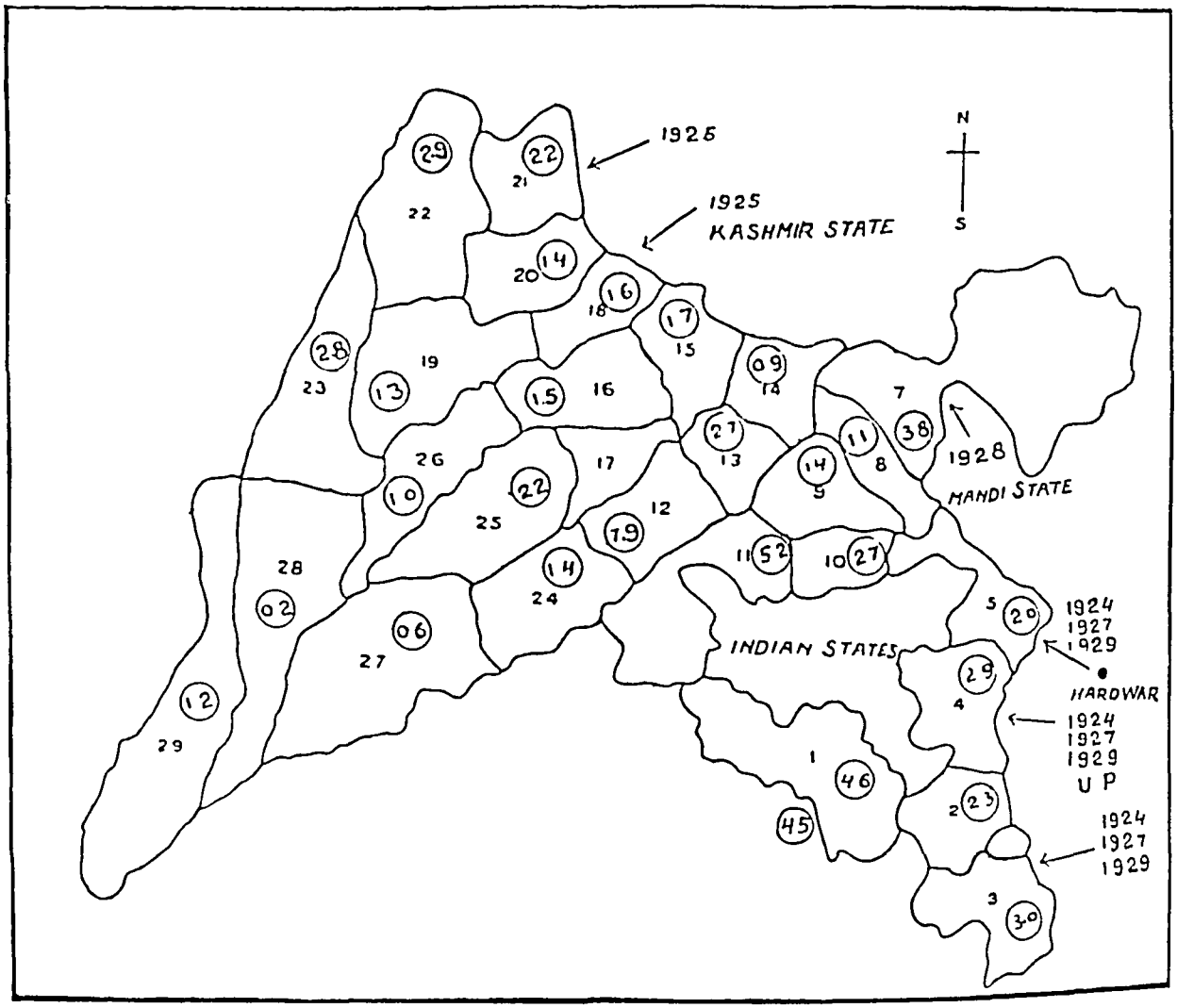
Cholera disappeared from the Punjab early in November 1924, and, so far as is known, from this time onwards until April 1925 the disease was completely absent from the province

The state of Kashmir which lies to the north of the Punjab is believed to have been infected by pilgrims from Hardwar in the summer of the previous year, in same manner as the Punjab, but in this state, although no conspicuous outbreak took place in 1924, a small number of cases continued to occur throughout the winter of 1924-1925, in spite of the fact that the country was under snow and the thermometer was several degrees below freezing point With the onset of spring (March) a definite recrudescence took place which was followed in the ensuing summer, by a severe and widespread epidemic in Kashmir in which over 10,000 persons lost their lives

The first outbreak of cholera in the Punjab took place in the north of the Punjab in a district (Jhelum) whose eastern boundary is co-terminous with that of Kashmir (*vide* Map) It was not possible to trace the manner in which cholera spread from Kashmir to the Punjab, but it was surmised, as stated elsewhere (Gill, 1926), that the small unreported outbreak of cholera that occurred at the town of Pind Dadan Khan in Jhelum district on April 1st, 1925, was due to importation from Kashmir It so happened that a religious fair, which is usually attended by some 50,000 persons, was about to be held at Katas–a village some 10 miles distant from Pind Dadan Khan–and subsequent investigation rendered it probable that infection was conveyed from Pind Dadan Khan to Katas The fair passed off without incident until the last day (April 12th) when, following the ceremonial immersion in and drinking of the waters of the sacred tank, an explosive outbreak involving several hundred persons broke out amongst the pilgrims who at that time were dispersing or about to disperse to their homes

As a result of this catastrophe cholera was spread by road and rail far and wide, but mainly to neighbouring districts, with the result that, within a fortnight, some 700 seizures and 320 deaths from cholera occurred (almost exclusively amongst pilgrims) in 11 districts of the province In most instances the disease was limited to pilgrims, but in a few localities the arrival of an infected pilgrim was followed by small local outbreaks, and in one village the discovery of a corpse of a pilgrim who had died from cholera in a canal distributary which formed the main source of drinking water of the village, was followed by a short but exceedingly sharp outbreak Some of the secondary

MAP OF THE PUNJAB



Explanation

Cholera death-rate per 10,000, 1900–1929
The arrows indicate main direction of spread
in the years indicated

DISTRICTS

1 Hissar
2 Rohtak
3 Gurgaon
4 Karnal
5 Ambala
6 Simla
7 Kangra
8 Hoshiarpur
9 Jullundur
10 Ludhiana
11 Ferozepur
12 Lahore
13 Amritsar
14 Gurdaspur
15 Sialkot
16 Gujranwala
17 Sheikhupura
18 Gujrat
19 Shahpur
20 Jhelum
21 Rawalpindi
22 Attock
23 Mianwali
24 Montgomery
25 Lyallpur
26 Jhang
27 Multan
28 Muzaffargarh
29 Dera Ghazi Khan

foci gave rise to others in this manner the number of infected localities—each actively infective for a brief period only—steadily increased, so that within a month of the outbreak at Katas some 2,000 seizures and 913 deaths from cholera occurred By the end of May the disease began to decline, but small outbreaks continued to occur mainly in the originally infected districts until the month of October, when the disease spontaneously disappeared

In May, however, cholera was imported into several districts in the south-east of the province by pilgrims from Hardwar and other places in the United Provinces and, as a result of infection derived from this source, a series of small outbreaks occurred in the districts of Gurgaon, Ambala and Hoshiarpur during the months of June to September

The total number of cholera deaths during the year was 3,049 (0 15 *per mille*) and the 634 infected localities comprised 49 towns and 585 villages The mean number of deaths in infected towns and villages was 11 7 and 4 2 respectively, whilst the urban and rural cholera death-rate was 0 28 *per mille* and 0 13 *per mille* respectively In the 49 infected towns, the number of deaths was 5 or less in 28 towns, in 12 towns between 6–20 deaths, in six between 21–50 deaths, and in 3 towns between 51 and 100 deaths, but in no large town did the outbreak exhibit explosive characters In its main features therefore the outbreak in 1925 differed sharply from the outbreak of the previous year The probable source of infection was Kashmir in the north and not Hardwar in the south-east, the area mainly involved was likewise distinct and so also was the period of the year of the main outbreak—(May) instead of August or September—and finally the conspicuously explosive character of the original outbreak at the Katas fair was in sharp contrast with the slow and insidious onset of the epidemic in the previous year

1926

After the disappearance of cholera in October 1925 the province remained completely free until April 1926, when two deaths occurred, which were followed by 3 in May and 5 in June The source of infection could not be traced but it is perhaps significant that they did not occur in localities infected at the end of the previous epidemic, but in the area which is usually infected by pilgrims from Hardwar The chief feature of the year was the failure of the disease to spread and the absence of any outbreaks of appreciable proportions The total mortality during the year was 87 and in the 11 infected towns the mean number of deaths was 3 9, whilst in the 23 infected villages the mean number of deaths was 1 9

In 8 out of the 11 infected towns the number of deaths was five or less, and in the remaining three, the cholera deaths were less than 20 The mild incidence of cholera may have been due to decreased importation, as the cholera death-rate in the United Provinces was 0 13 *per mille* as compared with 0 17

per mille in the previous year, but it is doubtful whether this circumstance was wholly responsible for the low incidence of the disease

1927

The feeble outbreak of cholera in 1926 ended in November and the province was completely free, so far as is known, until April 1927, but in this month a Kumbh Fair was held at Hardwar and the re-appearance of cholera in the Punjab followed closely upon the return of pilgrims to the Punjab The culminating days of the fair were April 14th and 15th and within a week of the latter date some 100 cases and 48 deaths occurred (mostly amongst pilgrims) in 15 districts In some instances cholera declared itself during the return journey, but more often shortly after arrival, in others, a week or ten days elapsed before the disease developed, and in still others the pilgrims remained healthy whilst their relatives and friends fell victims to the disease

By the beginning of May the vast majority of pilgrims had reached their homes and the history of the epidemic during this month comprised a trail of small outbreaks which for the most part lasted only 3 or 4 days During June the number of infected localities, as well as the number of seizures in each, steadily increased, and in July, August and September a widespread epidemic, presenting similar features to those exhibited in the year 1924, occurred In a few towns and villages explosive outbreaks took place Thus, in Okara town, 118 seizures and 37 deaths occurred within the space of 48 hours, as the result of the infection of a well by a wandering faqir whose dead body was found near its mouth Of an entirely different nature was the outbreak in Kasur town, which will be described in detail later It will suffice to state here that Kasur experienced a prolonged epidemic which was responsible for 6,324 seizures and 330 deaths Other towns in which epidemics of the same type occurred were Lahore, Amritsar, Moga and Pattoki

The total cholera deaths during the year were 11,286 (0 55 *per mille*) The number of infected towns was 76 and the mean number of deaths in each was 18 8, whilst in the 1,183 infected villages the mean number of deaths was 7 6 The urban and rural death-rate was 0 67 *per mille* and 0 54 *per mille* respectively, the relatively high death-rate in rural areas being mainly due to spread of infection from Kasur town to a large number of villages in the Lahore and Ferozepore districts

Of the 76 infected towns, in 42 the number of deaths was five or under, in 23 towns the deaths varied between 6 and 20, in five, between 21–50, in three between 51–100, and in three towns over 100 deaths occurred

In its main features the outbreak closely resembled that of the year 1924, with the exception that in 1927 a Kumbh Mela was held at Hardwar and the number of cholera-infected pilgrims was in consequence exceptionally large As in 1924, Hardwar was practically the sole source of infection and, as in 1924, the epidemic involved almost precisely the same area The seasonal periodicity was almost identical in the two years and so likewise was the

character of the individual outbreaks—a few explosive in character and circumscribed in area and a large number prolonged in duration and widely diffused in distribution

1928

The widespread epidemic which followed the Hardwar fair in 1927 came to an abrupt conclusion in October and as usual, the province remained, so far as is known, completely free from infection until the month of March 1928 But at the end of March something unusual occurred On March 31st cholera broke out (36 seizures and 26 deaths) at Jogindar Nagar in Mandi State—a Himalayan State which, so far as is known, had not previously been infected for many years This outbreak was shortly followed by another (28 seizures and 18 deaths) at Baij Nath in Kangra district, but a more serious development was the appearance early in the month of May of cholera in the Kulu Subdivision of the Kangra district, which has only once (1892) been infected during the past 60 years The first case occurred in Kulu (Sultanpur) on May 4th and from this date until May 17th an average of 2 seizures a day occurred, and this state of affairs continued without material change until May 23rd, when cholera suddenly and almost simultaneously appeared in epidemic form in the town of Kulu (Sultanpur) and in many, including even the most inaccessible, villages in the Kulu tahsil The epidemic reached its maximum during the third week in June and thereafter it subsided after causing 1,746 seizures and 1,164 deaths The early history of this outbreak is wrapped in obscurity, but it would appear that cholera was introduced into Mandi State by pilgrims returning from the Hardwar fair in the previous year and that sporadic cases continued to occur in a few villages in this State throughout the winter It would thus seem that the happenings in Kashmir in the winter of 1924-1925 were repeated precisely in another Himalayan State in the winter of 1927-1928 and that, in both cases, Hardwar was the *fons et origo mali* It is certain that, without recent importation, cholera appeared in the spring in Mandi State and, although no cases of cholera are known to have been imported into Kulu, it is known that the alarm occasioned by the outbreak of cholera at Jogindar Nagar led to the flight of many of the residents of Jogindar Nagar in the Kulu Subdivision

The character of the outbreaks in the Kulu valley varied At the commencement and for a period of nearly 3 weeks a few sporadic cases alone occurred, but later the epidemic was frequently explosive in type, whilst towards the end the outbreaks were smaller and more protracted in duration Entirely distinct from this northern epidemic, which was strictly confined to Kangra district (in which 1,447 or nearly 71 per cent of the total mortality of the year took place) a small number of outbreaks (18 deaths) occurred in April and May as the result of importation from Hardwar and other places, which were followed in June and July by a number of minor epidemics, without explosive characters, in several of the central and south-eastern districts of the Punjab The highest mortality, as in 1925, occurred in June, but the

disease disappeared early in October Contrary to precedent, a fresh wave of importation occurred in November consequent upon the collection of some 700,000 pilgrims at Thanesar for the Sun Eclipse Fair A detailed account of this outbreak will be given later and it will therefore suffice to state that it gave rise to 11 seizures before the fair (importation), 38 seizures during the fair (local), and 84 seizures in the Punjab and 119 seizures in the United Provinces, after the fair

The total mortality during the year was 2,034 (0 10 *per mille*), and the urban death-rate was 0 07 *per mille* and the rural death-rate 0 10 *per mille* The mean number of deaths from cholera in the 40 infected towns was 3 9, whilst in 215 infected villages the mean number of deaths was 8 7 Contrary to custom the rural exceeded the urban death-rate, which is due to the fact that the area mainly involved (Kulu) is almost devoid of towns No town was seriously infected and in the 40 infected towns the number of deaths was five or less in 30, whilst in the remaining 10 towns the number of deaths was 20 or less

This epidemic therefore presented many unusual features In the first place the main source of infection was a Himalayan State, secondly, the epidemic mainly involved an area in the north of the province which had never been infected since 1892, which, like the year 1928, was one year after a Kumbh Fair was held at Hardwar, thirdly, the epidemic reached its maximum in June, instead of August or September, and fourthly, towns were rarely infected and the rural death-rate, in consequence, exceeded the urban death-rate

1929

After the usual quiescent period during the winter, reports were received from a number of districts, more especially Gujrat, Jhelum, Shahpur and Mianwali, during March, April and May, of the occurrence of isolated cases of cholera preceded or accompanied by widespread outbreaks of diarrhœa in the infected villages This occurrence–unusual in recent years but often recorded in the past–was thought to presage a severe and widespread outbreak during the ensuing cholera season In the event it did not do so, but a moderately severe epidemic, apparently unassociated with recent importation, occurred in many of the central and northern districts during the months of May and June In the latter month, as the result of recent importation from the United Provinces, a number of outbreaks occurred in several districts in the south-east and east of the province, more especially Gurgaon, Ambala, Karnal, Hoshiarpur, Jullundur, Lahore and Amritsar, and, as a combined result of infection derived from these two sources, outbreaks occurred widely throughout the province during the months of July, August and September

These outbreaks, with the exception of one explosive outbreak in Lyallpur [illegible]t, which will be described later, and an outbreak which caused 123 deaths As in [illegible] city, were mainly of the protracted type and the only feature call- 1924, the ep- [illegible] was the relatively high incidence of the disease in urban periodicity was a

areas, which was mainly due to the occurrence of outbreaks of several weeks' duration or months in four towns whose sanitary arrangements are peculiarly defective The urban death-rate was 0 35 *per mille* or more than four times the rural death-rate (0 08 *per mille*) In most cases, however, the outbreaks were small in extent and short in duration, and in 44 out of the 66 infected towns, the number of deaths was five or less, in 11 towns between 6–20 deaths, in 7 towns, between 21–50, in two towns between 51–100, and in two towns over 100 deaths

The main statistical data in regard to the incidence of the disease in the province during the years 1924–1929 are summarized in Table I

TABLE I

Cholera statistics, Punjab, 1924–1929

Year	Cholera death-rate	Towns			Rural areas		
		Urban death-rate	Number of infected towns	Mean number of deaths in infected towns	Rural death-rate	Number of infected villages	Mean number of deaths in infected villages
1924	0·16	0·44	56	16 4	0·13	504	4 8
1925	0 15	0·28	49	11 7	0·13	585	4 2
1926	0·004	0 02	11	3·9	0 002	23	1 9
1927	0·55	0 67	76	18·8	0 54	1,183	7·6
1928	0·10	0·07	40	3·9	0·10	215	8·7
1929	0·11	0·35	66	11 8	0 08	407	3 7
Mean	0·18	0·30	49·7	11 1	0 16	486	5 1

From a scrutiny of Table I it will be seen that whilst in no year was the province free from cholera, in the year 1927, following a year in which death-rate was exceptionally low, an epidemic of considerable magnitude occurred in association with a Kumbh Fair at Hardwar It will also be observed that the mean urban death-rate during the period under review was nearly double the rural death-rate, and that in the year 1928 alone (in which the main epidemic area contained no large towns) did the rural death-rate exceed the urban death-rate

It may also be added that the mean number of infected towns (49 7) represents approximately 27 per cent of all towns, whilst the corresponding figure in the case of villages is 1 4 per cent The mean number of deaths in infected towns (11 1) is also more than double the mean number of deaths in infected villages (5 1), from which it may be inferred that not only are towns relatively more frequently infected, but the individual outbreaks in towns are relatively large

The varying intensity of cholera epidemics in urban areas is shown in Table II, from a scrutiny of which it will be seen that in 61 per cent of the 298 infected towns, cholera occasioned only five deaths, whilst in 26 per cent the number of deaths was between 6–20, in 7 per cent, between 21–50 deaths, in 3 per cent between 51–100 and in 3 per cent over 100 deaths The seventeen urban outbreaks in which the number of deaths exceeded 50 were, with few exceptions, protracted in duration and out of the 298 outbreaks in towns, only eight (2 7 per cent) exhibited definite explosive characters, whilst out of 2,917 outbreaks in villages approximately 60 (2 per cent) exhibited well-marked fulminant features

TABLE II

The incidence of cholera mortality in towns

Year	5 deaths and under	6–20 deaths	21–50 deaths	51–100 deaths	Over 100 deaths	Total number of infected towns
1924	30	18	4	1	3	56
1925	28	12	6	3	Nil	49
1926	8	3	Nil	Nil	Nil	11
1927	42	23	5	3	3	76
1928	30	10	Nil	Nil	Nil	40
1929	44	11	7	2	2	66
TOTAL	182	77	22	9	8	298
PERCENTAGE	61	26	7	3	3	100

In Table III the epigraphical features in respect of cholera mortality in the Punjab during each of the years 1924–1929 is shown, together with the mean cholera death-rate per 10,000 of population in each district of the province during the period 1900–1929 In the years 1924 and 1927 the incidence of the disease was relatively high in the districts in the east and south-east of the province from which the majority of the pilgrims to Hardwar hail, and this distribution may be regarded as typical of what may be termed, for convenience, a 'Hardwar epidemic' In 1925 cholera mainly affected the three northern districts of Shahpur, Gujrat and Jhelum, and this unusual distribution, it has been shown, was occasioned by an explosive epidemic in Jhelum district in the north of the province, which appeared to be due to infection derived from Kashmir The small epidemic in 1926 appears to conform in distribution to the Hardwar type In 1928 the brunt of the epidemic was experienced by the Kulu Subdivision of the Kangra district and this circumstance, it has been shown, was

associated with the spread of infection from an adjacent Himalayan State (Mandi)

In 1929, there appeared to be two epidemic areas, the earlier being apparently associated with the recrudescence of the disease in certain of the central and northern districts, whilst the latter, which was due to importation from the United Provinces, showed the usual distribution associated with infection derived from the south-east

TABLE III

Cholera mortality by districts in the Punjab, 1924–1929

	Districts	1924	1925	1926	1927	1928	1929	Total	Mean death-rate from cholera per 10,000 of population (1909–1929)
1	Hissar		2		488	5	6	501	4·6
2	Rohtak				220	53	36	309	2·3
3	Gurgaon	6	381	2	92	55	247	783	3·0
4	Karnal	5			195	13	86	299	2·9
5	Ambala	51	68	11	168	100	73	471	2·0
6	Simla				1			1	1·0
7	Kangra	30		3	10	1,447	3	1,493	3·8
8	Hoshiarpur	14	77	2	194	27	81	395	1·1
9	Jullundur	28	58	1	225	21	70	403	1·4
10	Ludhiana	56	65	0	401	4	56	582	2·7
11	Ferozepore	224	3		3,396	15	52	3,690	5·2
12	Lahore	1,397	53	7	4,070	35	456	6,018	7·9
13	Amritsar	191	66	4	652	39	234	1,186	2·7
14	Gurdaspur	117	115	17	117	5	103	474	0·9
15	Sialkot	399	63		17	10	162	651	1·7
16	Gujranwala	44	71	33	6	11	51	216	1·5
17	Sheikhupura	9	43		153	22	72	299	0·8
18	Gujrat	81	276	1	19	5	45	427	1·6
19	Shahpur	35	875		1	10	2	923	1·3
20	Jhelum	1	398	3	1	23	17	443	1·4
21	Rawalpindi	50	93	1	20		33	197	2·2
22	Attock	5	6		1		9	21	2·9
23	Mianwali		14			6	92	112	2·8
24	Montgomery	201	8		502	107	29	847	1·4
25	Lyallpur	378	99		261	4	280	1,022	2·2
26	Jhang	22	185		3	1	2	213	1·0
27	Multan	5	30		26	2	9	72	0·6
28	Muzaffargarh	2		2	43	12	3	62	0·2
29	D G Khan				4	2		6	1·2
	TOTAL	3,351	3,049	87	11,286	2,034	2,309	22,116	

In the Map of the Punjab is shown the apparent source of infection and direction of spread of cholera during the six years 1924–1929, together with the mean annual cholera death-rate of each district per 10,000 of population during the thirty years 1900–1929 The figures are of a composite nature, but when the facts recorded in the annual public health reports of the province

are interpreted in the light of the events during the six years 1924–1929, it would seem that the province may be divided from the point of view of the spatial distribution of cholera, into three areas, viz, (1) a large epidemic area in the south-east and east of the province, specially liable to be infected from Hardwar, (2) an area in the north, specially liable to infection from the Himalayas, and (3) an area in the south-west which is scarcely ever attacked by cholera

In Table IV the monthly incidence of cholera mortality during each of the years 1924–1929 is shown —

TABLE IV

The seasonal incidence of cholera mortality, Punjab, 1924–1929

Year	January	February	March	April	May	June	July	August	September	October	November	December
1924		1	1	4	18	38	676	1,216	1,275	118	4	
1925				426	1,013	716	223	271	340	59	1	
1926				2	3	5	26	22	26	3		
1927			2	48	352	1,791	6,592	2,019	422	60		
1928			4	5	96	914	703	176	82	11	30	13
1929	1		2	21	132	490	259	494	708	172	22	9
Total	1	1	9	506	1,614	3,954	8,479	4,198	2,853	423	57	22
Mean			1·5	8·4	26·9	65·9	141·3	69·96	47·55	7·05	0·95	0·36

It will be seen that epidemics of Hardwar origin (1924 and 1927), although due to importation in April and May, are slow in onset and do not attain their maximum intensity until July, August or September On the other hand epidemics associated with infection derived from Himalayan States (1925 and 1928) not only exhibit a northern distribution, but commence earlier and attain their maximum intensity in May or June and thereafter decline In the year 1929, when there appeared to be two epidemic areas, the epidemic in the north reached its maximum in June, whilst the epidemic associated with importation from the United Provinces reached its maximum in September, there were thus two peaks in this year instead of the more usual single peak in August or September

(*b*) *Detailed description of typical epidemics*

It has been shown that there are two sharply defined types of cholera epidemics, viz, protracted epidemic, slow in onset, insidious in course and

diffused in distribution, and the explosive epidemic, which is short, exceedingly sharp in intensity and circumscribed in distribution

The usual type, both in towns and villages, is the protracted epidemic and in so far as the period 1924–1929 is concerned, nearly all extensive outbreaks in towns were of this type

A typical explosive epidemic occurred at the Katas Fair in April 1925 and other examples of fulminant outbreaks of short duration occurred at Okara town in 1927 and in a village in Lyallpur district in August 1929, but, as already stated, out of the total of 298 outbreaks in towns only eight (2 7 per cent) exhibited definite explosive characters, whilst out of 2,917 outbreaks in villages, approximately 60 (2 per cent) exhibited well-marked explosive features

It is now proposed to give a detailed account of examples of these two types of epidemics and also of a typical epidemic, unassociated with explosive characters, at a large religious fair

(1) The explosive type of epidemic

On August 22nd, 1929, a man, who was seized with cholera whilst travelling from an infected village to his home in Lyallpur district, entered an uninfected village (Chak No 282 G B) (population 1,160) in search of medical aid He died on August 24th and after his death his clothes were washed at the well which forms the main water-supply of the village On August 25th a sudden and violent outbreak of cholera occurred amongst the inhabitants of the village with the result that 180 seizures and 16 deaths occurred before midnight on the 25th It so happened that a marriage-party halted at the village on the 24th and they also drank water from the well On August 25th and 26th 21 seizures and 9 deaths occurred amongst this party in five other villages, whilst the daily number of seizures and deaths amongst the inhabitants of the village was as follows —

	Seizures	Deaths
August 25th	180	16
„ 26th	3	29
„ 27th	10	8
„ 28th	0	7
„ 29th	4	9
„ 30th	2	6
„ 31st	0	3
September 1st	1	6
„ 2nd	0	6
Total	200	90

(2) The protracted type of epidemic

The epidemic which occurred in Kasur town (population 31,018) in the summer of the year 1927, constitutes a typical example of a prolonged and severe outbreak in a town

Kasur, which is the headquarters of the Kasur Subdivision of the Lahore district, is a thriving commercial town possessed of good communications by road and rail The town presents the features common to all old Punjab towns–a central congested nucleus of many-storied brick buildings and a number of detached hamlets (the relics of fortified posts) which now constitute a suburban area of one-storied brick houses and mud huts From the public health point of view Kasur has long earned the unenviable reputation of being one of the most insanitary towns in the province It has no municipal water-supply, no drainage system worthy of the name and its conservancy arrangements are exceedingly primitive It has suffered more frequently and more severely from cholera than almost any other town in the Punjab During the last six years an epidemic which occasioned 145 deaths occurred in the year 1924, the outbreak in 1927, which is about to be described, was responsible for 330 deaths, and a smaller outbreak (46 deaths) occurred in the year 1929

It is necessary to describe the conservancy arrangements in some detail as they appeared to exercise an important bearing upon the cholera epidemic in the year 1927 Scattered throughout the town are 18 official and innumerable unofficial filth depôts, which consist of open enclosures in which the municipal scavengers dump the night-soil collected from the adjacent houses, streets and lanes From these depôts the night-soil is removed in open country carts by contractors who pay the Municipal Committee for the privilege

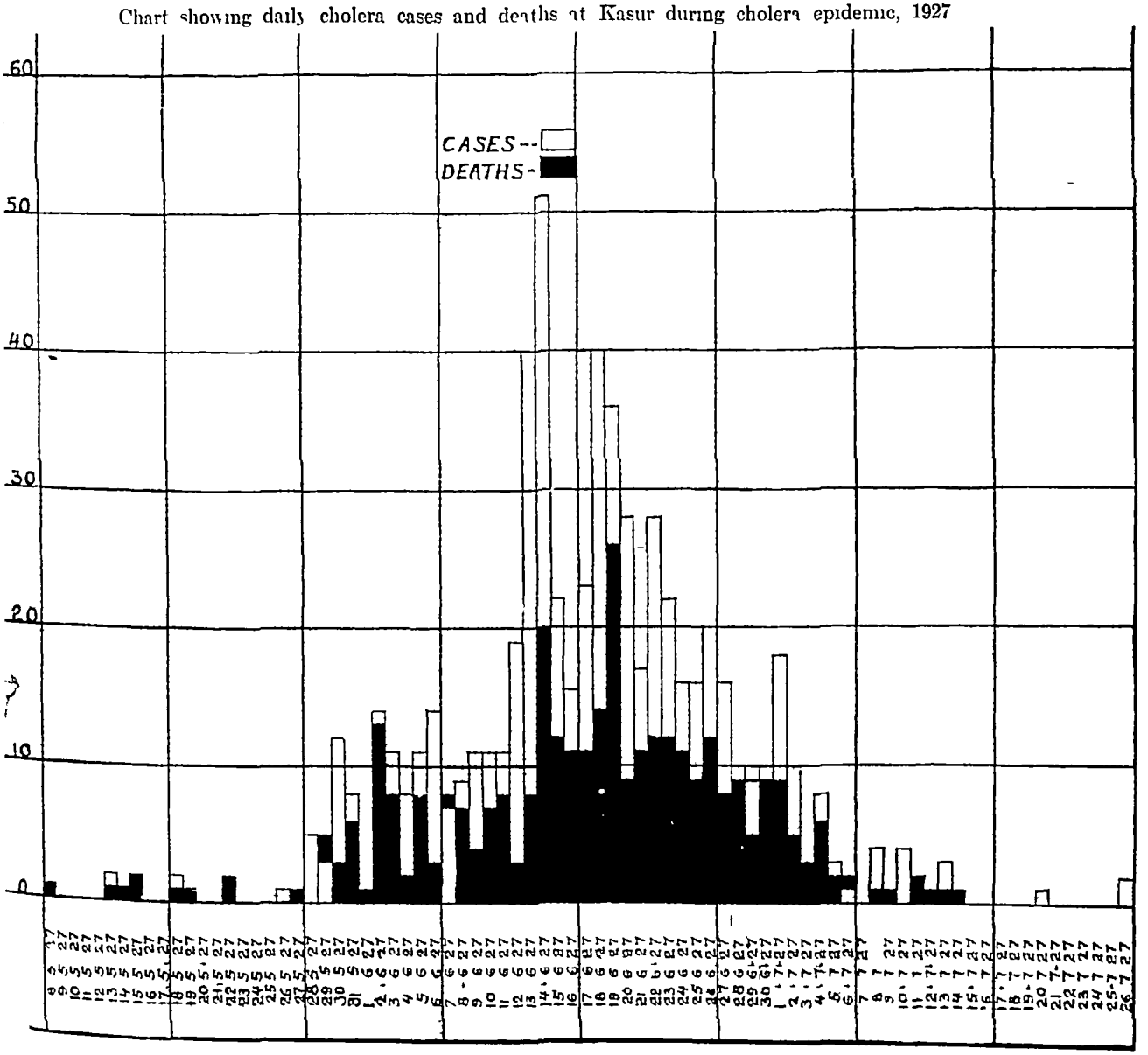
The contractors recoup themselves by selling the night-soil to the cultivators who use it for manuring their fields Two circumstances combine to render the working of this extremely defective system of conservancy peculiarly inefficient In the first place when the cultivators do not require manure, there is no longer any incentive to the contractor to remove it from the town, in which, in consequence, it accumulates Secondly, disputes often arise between the contractor and the Municipal Committee and when this happens the contractor ceases work until the dispute is settled An incident of this nature occurred in April 1927 and no night-soil was, in consequence, removed from six filth depôts from April 1st to June 20th, 1927 It thus came about that some of these dumps, at the time of the cholera epidemic, contained several hundred tons of human ordure

It may be added that during the epidemic it was observed that cholera stools were frequently thrown upon these dumps The water-supply of the town is derived from wells–786 in number–which are evenly distributed all over the town The origin of the outbreak of cholera could not be traced, but, following the return of pilgrims from Hardwar in April, a number of cases of cholera occurred in neighbouring villages The first suspected case of cholera

in Kasur town occurred on May 8th, and on May 13th two seizures were reported from another part of the town The fourth case occurred in another locality on May 14th There were 2 seizures on the 15th and two on the

CHART 3

Chart showing daily cholera cases and deaths at Kasur during cholera epidemic, 1927



18th These eight cases–all regarded as 'suspected' and not reported–occurred in five separate parts of the town in a manner suggesting the presence of several distinct foci of infection

From May 18th–21st there were no further seizures, but on the 22nd there were 2 seizures, then, after an interval of four days, one seizure was reported On May 28th there were five seizures and three on the next day Up to this time 19 seizures occurred during a period of 22 days On May 30th, 12 seizures took place and on this day the epidemic may be considered to have commenced The daily number of seizures and deaths throughout the epidemic is depicted in Chart 3 from a scrutiny of which it will be seen that during the first 10 days of June the daily number of cases ranged from 7–14 The Id festival commenced on the 11th June and the festivities in connection therewith lasted until June 14th, on which date the number of seizures rose to 51 Thereafter the daily number of cases declined slowly but steadily, and in the middle of July the epidemic came to an end

The outbreak which was responsible for 634 seizures and 330 deaths thus lasted for a period of 11 weeks–May 8th to July 26th–whilst definite epidemic conditions prevailed for 34 days (May 30th to July 2nd) In this epidemic all parts of the town were not equally infected, the disease being most intense in the area inhabited by the poorer section of the population where the five dumps from which the filth had not been removed for several weeks are located In this area the occurrence of more than one case at a time in any one house was unusual, and, although few households escaped, in many streets the scattered distribution of the cases suggested a chance distribution and a widely disseminated source of infection The harm wrought by this epidemic was, however, not confined to the town of Kasur, since infection was carried from Kasur to several towns and to 48 villages in Lahore and Ferozepore districts where 781 seizures and 509 deaths took place These villages in their turn infected others and it thus comes about that Kasur was, directly and indirectly, mainly responsible for the fact that 7,466, out of a total of 11,286 deaths from cholera in the province during the year 1927, occurred in the Lahore and Ferozepore districts

The chief features of the outbreak were, in the first place, the failure to discover the source of infection and the mode of spread of the disease, and, secondly, the failure of all efforts to control the outbreak or to check its progress In one instance in which 8 seizures occurred in a single house it was suspected that a water-carrier had infected the water-supply of the household In six instances in which 3 or more cases occurred at short intervals in a single family direct spread from the sick to the healthy was suspected Attempts to connect the cases with milk, fruit (melons), vegetables, ice and aerated waters all failed The scattered nature of the cases, as well as the fact that from the commencement of the epidemic the wells in the vicinity were systematically disinfected, appeared to render it improbable that

water was largely concerned in the spread of the disease Dust-storm preceded a small rise in the daily number of cases on two occasions and it was thought that food-stuffs may have been infected in this manner The sharp rise in the number of seizures on June 14th gave rise to the suggestion that the feasting associated with the Id festival was indicative of the spread of infection by food and drink Finally, it was surmised, on the basis of the observation that the incidence of the disease was peculiarly high in the vicinity of the fly-infested filth dumps, that flies were responsible for spreading the disease and Dr A B Aiola, D P H, Assistant Director of Public Health, Lahore Circle, who conducted the inquiry, opined 'that the vicious circle established for the continued infection of flies was responsible for the disastrous proportions of the epidemic'

(3) Cholera epidemics at fairs

The outbreak at Katas Fair in 1925 was fortunately the only example of an explosive epidemic at any religious fair in the Punjab during the period under review As an illustration of the more usual character of cholera outbreaks at religious fairs, which by reason of their great importance and of the fact that they cannot strictly be regarded as either a protracted outbreak or as an explosive epidemic, it is proposed to give a brief summary of the outbreak of cholera at Thanesar in November 1928 on the occasion of the Sun Eclipse Fair This fair which was attended by some 700,000 pilgrims commenced on November 7th, 1928 and ended on the 15th, on which date, during the eclipse of the sun, the pilgrims indulged in a ceremonial ablution in the waters of the sacred tanks

At the time of the fair the Punjab was completely free from cholera, but the disease still prevailed in parts of India from which pilgrims were expected to come The disease was therefore most likely to be introduced by these pilgrims, but, whilst their number is not accurately known, the number of arrival by rail was 245,439 All the pilgrims, including the arrivals by road, were medically inspected before being permitted to enter the fair area at railway and road inspection posts established at numerous points on the perimeter of the fair Pilgrims from outside the Punjab were in fact solely responsible for the infection of Thanesar On November 7th two pilgrims hailing from Burma, who had stopped at Gaya–a cholera infected area–were found on arrival to be suffering from cholera and were removed from the railway station to the Infectious Diseases Hospital The other imported cases comprised two pilgrims hailing from Almora, who came to Thanesar via Hardwar and reached Thanesar on the 8th, one each from Benares and Sitapur in the United Provinces, and two from Hoshangabad in the Central Provinces on the 9th, and one from Gaya (Bihar and Orissa) on the 10th In these cases cholera declared itself either before or shortly after arrival at Thanesar or

within 24 hours of taking up their abode in the fair The incoming railway traffic ceased abruptly on the 11th, up to which time eleven cases of cholera, in all of whom infection was probably acquired outside the Punjab, were detected On the 11th November 14 persons developed cholera in the fair area–two in Block 4, six in Block 9 four in Block 3, and one each in Blocks 2 and 7–and as seven of these cases were Punjabis hailing from neighbouring uninfected localities, it is assumed that they acquired the disease locally On the 12th there were 8 seizures, 5 on the 13th, 9 on the 14th, and 2 on the 15th–making in all 38 seizures between the 11th–15th November

By the 15th November Thanesar was deserted, but during the return journey 64 pilgrims developed cholera on the way to their homes in the Punjab–and in this manner 36 localities (in which 20 indigenous cases occurred) were infected In addition 119 cases occurred amongst pilgrims in the Saharanpur, Muttra, Almora and Naini Tal districts of the United Provinces

This report is not concerned with the medical and public health arrangements, which were made and carried out under the personal supervision of the senior author (C A G), to prevent the outbreak of cholera at this fair–a detailed account of which is given elsewhere (Gill, 1929) nor is it proposed to consider here the practical lessons taught by this epidemic, but it is necessary to draw attention to the striking contrast between the 49 widely scattered cases that occurred at Thanesar amongst several lakhs of people during a period of 7 days with the explosive outbreak at Katas Fair in 1925, when several hundred cases occurred within the space of 24 hours

III Discussion

It is not expedient to put forward, on the basis of this epidemiological investigation, anything more definite than provisional conclusions and the main object of this inquiry was to explore the field with a view to ascertaining the kind and nature of the problems that call for investigation by the more exact method of experimental research

With the proviso therefore that all conclusions reached are at present tentative, it is proposed to consider the implications which the observations recorded in the previous pages appear to justify

Endemic home—It is clear that there is still substantial ground for the belief that cholera is not *permanently* endemic in any part of the Punjab The view that importation from the United Provinces, and more especially *from* Hardwar, is directly and indirectly the predominant factor in causing outbreaks of cholera in this province is also confirmed These epidemics, which for convenience have been called Hardwar epidemics, possess in fact, distinctive characters both in respect of the persons primarily involved (Hindus) and in the area (the districts in the plains of the Ambala, Lahore and Jullundur Divisions) in which they mainly occur

But it is also clear that the Punjab is sometimes infected from the north and that outbreaks of cholera due to importation from the montane tract of the Himalayas are apt to occur more especially in the sub-montane districts of Rawalpindi Division and in the sub-montane tracts of the Jullundur Division Cholera, however, would not appear to be permanently endemic in any part of the Himalayan region, but the available facts appear to suggest that cholera sometimes persists throughout the winter in the Himalayas and the northern districts of the Punjab and then breaks out in epidemic form in the following spring There would thus appear to be a temporary form of endemicity, which although lasting for one winter only, is capable of causing a widespread epidemic mainly in the northern half of the Punjab in the following summer

Reservoir of infection—The available facts lend support to the conclusion reached by Sufanjam Khan (1929) that persons suffering from or who recently recovered from cholera are alone concerned in the spread of the disease It is not suggested that 'chronic carriers' do not occur but it is held that the epidemiological observations made in the Punjab during the past six years are consistent with the view that 'acute carriers,' i e, persons in the incubation stage, convalescents and recent contacts, are alone concerned in the spread of the disease In this connection it may be remarked that the 'carrier' who was responsible for the epidemic of cholera in Puri jail which was investigated by E D W Greig (1913) had only recently—about 2 weeks previously—recovered from an attack of cholera

Seasonal periodicity—The clear recognition of a northern source of infection and a northern type of epidemic appears to clear up a puzzling point in connection with the seasonal periodicity of the disease The seasonal character of most epidemics, in any given area, is usually constant, but in the case of cholera, it has been shown that epidemics may occur at any period of the year between April and November S M Jacob (1923) has also shown, as the result of a statistical analysis of the cholera mortality in the Punjab, that during the 30 years from 1867–1896, the disease exhibited two maxima, the first in the months of May and June and the second in September, so that the chart exhibits a double hump, whilst in the 30 years from 1897–1921, the mortality reached its maximum in September, with the result that the chart exhibits a single peak

It has, however, been shown that epidemics due to importation from the north are mainly restricted to the months of April, May and June, whilst epidemics dependent upon infection derived from Hardwar usually attain their acme in August or September It seems probable therefore that the double hump in the chart for the period 1867–1896 merely indicates that during this period northern epidemics were more frequent or more severe than they were during the second period of thirty years A scrutiny of the annual public health reports lends support to this view, and it would appear that the double hump in the period 1867–1896 is largely due to the high death-rate occasioned

by the great epidemic in the year 1892 which was partly northern in distribution and in which 61 1 per cent of the total deaths from cholera (over 75,000 in number) occurred in the months of May and June

The transmission factor —One of the most striking features brought to light in the foregoing pages is the distinctive features exhibited by the protracted and the explosive type of epidemic, and scarcely less striking is the remarkable difference in frequency between these two types of epidemics It is possible that the widely different character of these epidemics is dependent upon the varying virulence of the specific parasite, and that an explosive epidemic is due to infection with an organism of 'exalted' virulence Alternatively, it is possible that these two types of epidemics may be associated with different modes of transmission of the cholera vibrio In regard to the former hypothesis, it is impossible to express any opinion, but in regard to the latter, several considerations point to the conclusion that a difference in the mode of transmission of the specific parasite may be the factor of significant importance in determining the type of epidemic The epidemiological evidence appears to be conclusive that explosive epidemics are usually dependent upon the recent and massive infection of water, but it is difficult, although not impossible, to explain the protracted epidemic on this basis

Ever since the classical report of Snow (*vide* Rosenau, 1927) on the outbreak of cholera associated with the Broad Street pump, water has been regarded as the chief if not the sole source of infection in cholera epidemics This outbreak was indeed a typical example of an explosive outbreak, since the vast majority of the seizures and no less than 259 deaths from cholera occurred on September 1st and 2nd, 1854, amongst persons who had drunk water from this well, but the point it is here desired to emphasize is that explosive epidemics, which there is reason to believe are usually water-borne, are distinctly rare in the Punjab (about 2 5 per cent of all outbreaks) and it would not therefore appear that water plays a predominant part in the spread of cholera in this province

It is, of course, not impossible that a protracted epidemic may be due, in part at any rate, to water-borne infection, but it is held that the epidemiological facts are not readily explicable on the basis of water being the common source of infection in this type of epidemic Water may be responsible for a protracted outbreak if it is infected slightly or intermittently or if, by reason of its temperature or its composition, it is inimical to the vibrio But in the case of towns, such as Kasur, where the drinking water is obtained from some 786 wells it is not easy, on the basis of water-borne infection, to account for the daily occurrence of a small number of cases scattered over a wide area And it is particularly difficult to do so when, as at Kasur, the wells were systematically disinfected–without any apparent effect in checking the disease–from the commencement of the epidemic Then again, if water was largely concerned in the spread of cholera it would be expected that towns provided with a piped water-supply would be less liable to cholera than others This

surmise indeed led to the inclusion in the provincial public health reports of India of a table showing the average annual death-rate from cholera in towns *before* and *after* the introduction of water-supply or drainage schemes or both

A scrutiny of these data in respect of Punjab towns shows that this anticipation has not been fulfilled and that no significant reduction in the cholera death-rate has followed the installation of piped water-supplies in these towns, in fact, taking the six largest towns, a small reduction in the cholera death-rate in the case of three is counterbalanced by an almost equal increase in the case of the remainder Epidemiological considerations therefore suggest that a water-borne vibrio plays an unimportant part in the causation of *protracted* epidemics It is of course possible that during the course of long-continued epidemics the infection of the household supply may occasion an outbreak with a familial distribution, but this type of outbreak is relatively rare as compared with isolated cases in numerous houses dotted apparently at random over the infected part of the town This kind of distribution also renders it difficult to regard direct spread from the sick to the healthy as playing an important part in protracted epidemics

It is less easy to appraise the part played by articles of food and drink, more especially milk, fruit, and sweetmeats, but it is clear that, whatever part they may play, they are innocuous until they have been contaminated by the cholera vibrio, and the problem therefore to be solved is the means or the method by which they become infected It is more especially difficult to believe that massive contamination of food or drink by contact, by dust or by flies can be the main factor in determining a diffused epidemic of prolonged duration because massive pollution, in the case of water, occasions an epidemic exhibiting entirely different characteristics It would, in fact, appear to be necessary to postulate the existence of some agency, whereby, in infected localities, articles of food and drink could be slightly but systematically infected And having regard to the fact that the cholera vibrio is a delicate organism which, so far as it is known, is not capable of surviving for a prolonged period outside the human body it is difficult to believe that a focus of infection in refuse and soil would be capable of existing for a protracted period It is more reasonable to suppose that some more suitable environment for this delicate organism during its extra-corporeal phase must exist, and it may therefore well be that, like many other human parasites, it has acquired the power of passing this vulnerable stage in its life-history in the tissues of an alternative host If this be so, the insect with which on account of its numbers and its habits, the cholera vibrio is most likely to have acquired a symbiotic relationship is the house-fly, and it thus comes about that, on purely epidemiological grounds, the hypothesis was formulated that the house-fly may act as a true 'carrier' of the cholera vibrio and that this insect is mainly concerned in the mechanism of protracted epidemics

The accuracy or otherwise of this surmise can only be determined by experiment, but before detailing the result of the experimental investigation,

it may be well to consider more fully the extent to which the hypothesis is capable of accounting for the epidemiological facts

In the first place it has been shown that, whilst all towns in the east and south-east of the province are more or less equally liable to be infected with cholera, the towns which are peculiarly liable to epidemic visitations are those in which the conservancy arrangements are exceptionally bad It is also clear that defective conservancy is conducive to fly-breeding and that if, as happened at Kasur, cholera stools are exposed on filth dumps, ample opportunities exist for flies to become infected Assuming the house-fly to be a carrier it is therefore easy to account for the fact that cholera usually prevails most severely in those towns and parts of towns in which the conservancy arrangements are peculiarly bad, and it also explains why the usual victims of cholera are the poor and thriftless, who, on this very account, are necessarily congregated in the insanitary slums located on the outskirts of towns There are many Kasur in the Punjab, but it is not a little remarkable that the conditions prevailing in an English town (Sunderland) at the time of the first recorded epidemic of cholera in England in 1831 should have resembled so closely those prevailing at Kasur in the year 1927

In regard to the epidemic at Sunderland, Creighton (1894), in his classical History of Epidemics in Britain, states —

> 'The first experience of Asiatic cholera on British soil brought out very clearly one character of the infection which was seen to attend it everywhere during the following year and has always attended it in every subsequent invasion of the disease The virus, for all its opportunities, showed a marked preference for, an almost exclusive selection of, the lowest and least cleanly localities, and a considerable preference for persons of drunken or negligent habits The focus of the cholera (at Sunderland) was the town moor—upon this open space was deposited and left to accumulate for weeks together, the filth from the narrow lanes and passages of the low-lying and crowded quarter at the seaward end of the parish to the south of the High Street Some of the streets occupied by the poorer classes consisted of old residences of the well-to-do now divided into tenements Certain streets had as many as a dozen or twenty common middens "let in" to the street fronts of houses and covered by trap-doors in which the domestic refuse and sweepings of the street were collected as a source of profit and sold at stated times to farmers for manure Most of the attacks happened in this low-lying part of Sunderland with a soil and foundations sodden with filth and houses overcrowded and badly ventilated and its residents subject to the alterations of excess and want'

Then again, a fly-borne outbreak would necessarily be slow and insidious in onset, since it is permissible to assume, on analogy with other insect-borne

parasites, that some time must elapse between the importation of the virus and its dissemination by flies It has been shown that, whilst the evolution of an explosive epidemic is a matter of about 24 or 48 hours, a week or more invariably elapses between the date of the first case and the commencement of a protracted epidemic The outbreak at Sunderland also exhibited this feature since a prevalence of cholera nostras, in association with a few cases of true Asiatic cholera in August or September 1831, preceded the epidemic which started on October 23rd and continued until January 9th, 1832

Again, if the house-fly is capable of acting as a true 'carrier,' it is not only possible to account for the special liability of insanitary towns and parts of towns to protracted epidemics of cholera, but it also provides an explanation of the distribution in time and place of individual seizures, which, incidentally it may be remarked, bears a strong resemblance to the spatial distribution of cases of bubonic plague For given an infected fly population, it is not difficult to understand that the infection of food-stuffs, by the inoculation of the cholera vibrio into a cup of milk, or on to a piece of meat, will be responsible for infecting, not the household, but only the actual consumer of the infected article

Finally, the house-fly hypothesis provides a reasonable explanation of the part played by climatic and meteorological conditions in the natural history of cholera

It has long been recognized that extensive epidemics of cholera only occur during the hot weather in those parts of India, such as the Punjab, in which there is a well-marked cold weather, and that the disease prevails throughout the year in those provinces exhibiting a tropical climate all the year round This observation is also applicable to other countries, and in England cholera epidemics of the protracted type took place mainly in the hottest months of the year (August and September) It is true, in rare instances, that outbreaks have occurred during the depth of winter in England, Scotland and Northern Russia Creighton (1894) thus states that on the afternoon and night of Christmas day in 1831 there was a sudden explosion at Gateshead where at nearly fifty points cholera broke out almost at the same instant It is necessary therefore to draw a distinction between explosive epidemics and protracted epidemics, and there is reason to believe that an *explosive* epidemic may occur anywhere and at any time irrespective of the season of the year, provided the water-supply becomes heavily infected, but it seems to be true that *protracted* outbreaks of appreciable magnitude are restricted to the summer and autumn in temperate climates The inference that may be drawn from this fact is that relatively high atmospheric temperature is a necessary factor in the mechanism of protracted cholera epidemics, and it is possible that this fact may indicate that the cholera vibrio is unable to complete its cycle of development in the tissues of the house-fly when the temperature falls below a certain figure

Then again, the effect of atmospheric temperature on the metabolism of the insect carrier requires to be taken into consideration, and it would not necessarily follow that the range of temperature favourable to the bionomics of this insect is identical with that required to enable the cholera vibrio to undergo a cycle of development in the tissues of its insect-host The part played by atmospheric humidity in the epidemiology of cholera must likewise be regarded from the standpoint of its effect upon the insect-carrier and upon the specific parasite during its residence in the body of the alternative host

The part played by the climatic factors of temperature and humidity in the epidemiology of cholera may therefore be closely similar to the rôle which these factors play in the epidemiology of malaria and it may well be that when the influence of the climatic elements has been investigated in the manner employed by the senior author (Gill, 1921 and 1928) in the study of epidemiology of malaria, it will become possible to reconcile the puzzling and sometimes conflicting conclusions reached on the basis of statistical analysis

It seems probable, for example, that an absolute humidity of not less than 0 400 inch, which Sir Leonard Rogers (1928) has shown to be essential to the occurrence of cholera in epidemic form, carries implications mainly connected with temperature, since it is only in the presence of relatively high temperature that the aqueous vapour in the atmosphere can attain a pressure of 0 400 inch It may therefore be that it is atmospheric temperature, rather than absolute humidity, that explains the seasonal incidence of the disease in the Punjab and is responsible for the spontaneous disappearance of cholera with the onset of the cold weather

Then again, conflicting views in regard to the relationship of rainfall to cholera, which the exhaustive statistical studies of A J H Russell (1925-26) have served to emphasize, can now in large measure be reconciled, since, if the essential factor is the presence of certain associated conditions of temperature and humidity, it is no longer difficult to explain why cholera epidemics are apt to occur in some places before, in others during and in yet others after the monsoon To conclude, it is held, as indeed was clearly and forcibly suggested by W C Ross (1928), (who, however, only regarded the house-fly as an accidental polluter of food and drink), that, not only does the house-fly hypothesis provide a reasonable explanation of the peculiar relationships of cholera epidemics with the climatic factors of temperature and humidity, but it also provides a means whereby many obscure features in the epidemiology of the disease become capable of comprehension

Nevertheless, the hypothesis must remain an unsubstantiated surmise until it has been proved, by means of experiments, that the house-fly is capable of playing the part attributed to it in the natural history of cholera

IV The laboratory investigations

The object of this part of the inquiry was to determine the fate of the cholera vibrio in the tissues of the house-fly It was desired in particular to

know whether any evidence could be deduced pointing to the conclusion that the cholera vibrio underwent a cycle of development in the house-fly and, if so, in what manner and in what part of its anatomy did this cycle of development take place In the event of any evidence in favour of this hypothesis being forthcoming, it was further proposed to determine in what manner the cholera vibrio leaves the body of the insect and what conditions of temperature and humidity are necessary to the dissemination of infection by the house-fly

It is unfortunately not possible at present to provide an answer to all these questions and attention has mainly been confined to determining whether the house-fly is capable of acting as an alternative host of the cholera vibrio

Before detailing the results of the experiments designed to throw light upon this question it may be well to refer briefly to the present position in respect to the part played by non-biting insects in general and flies in particular in the spread of disease and more especially cholera It would seem that non-biting insects have not hitherto been regarded as playing anything more than a mechanical or passive rôle in disseminating bacterial diseases G S Graham-Smith (1914), who has exhaustively studied this subject, thus states, in reference to non-biting insects, that 'so far as we know none of the disease-producing organisms they are capable of distributing, undergo developmental changes within them' So far as the house-fly is concerned, all the observations hitherto made appear to have led to the conclusion that the fly acts only mechanically, like a platinum loop, in transferring the cholera vibrio from *fomites* to the food and drink of man The investigations of Graham-Smith and others, which were mainly conducted with organisms of the enteric group, clearly demonstrated that flies may carry in their internal organs many pathogenic and non-pathogenic organisms for varying periods ranging from 6 to 31 days, but he found that the cholera vibrio differed from others since it did not survive for more than two days in laboratory-infected flies

According to Graham-Smith, Maddox (1885) was the first to carry out experiments on the relation of flies to cholera Maddox states that he found cholera vibrios in the fæces of infected flies but his technique was not entirely satisfactory Graham-Smith also states Sawtchenko fed flies on broth cultures and found vibrios in their fæces two hours later He also found vibrios in the intestines and he gained the impression that they multiplied in their bodies The same author states that Simmonds, after placing flies on the intestinal mucous membrane of persons who had died of cholera, and then transferring them to large flasks, made roll cultures of these flies, at intervals from five to ninety minutes, and obtained positive results Uffelmann also is stated by the same author to have allowed two flies to feed on a liquid gelatin culture of *V choleræ,* and to have made cultures from them one hour and two hours later, which yielded 10,000 and 25 colonies respectively He also demonstrated that flies infected in this manner could contaminate milk Graham-Smith also cites Tsuzuki as having shown that infected flies could

contaminate media over which they walked, and Chantemesse and Ganon as having isolated vibrios from flies 17 and 24 hours respectively after infection Graham-Smith, experimenting with old laboratory cultures, found that the vibrios quickly died on the legs and wings, and that even in the crop and intestine their numbers rapidly diminished, so that cultures made more than 48 hours after infection yielded negative results, whilst the fæces were infective for 30 hours L Rogers (1921) quotes Barber as stating that flies, cockroaches and red ants harbour the organism for from 24 to 48 hours and occasionally up to 72 hours

From these reports it would appear that the *V choleræ*, unlike other pathogenic organisms such as *B typhosus* and members of the *salmonella* group, disappear from the body of the fly within about 48 hours

The above experimental data thus point to the conclusion that under laboratory conditions flies are capable of harbouring the cholera vibrio for a relatively short time and that during this period they are capable of disseminating the vibrio in a mechanical manner

Examples of the recovery of *V choleræ* from flies caught in nature in the immediate neighbourhood of cholera-infected houses are fairly numerous Graham-Smith thus cites Simmonds (1892) as stating that he isolated vibrios from a fly caught in a post-mortem room where autopsies on cholera were being made and that Tsuzuki succeeded in isolating the vibrios from flies captured in an infected house in Tientsin The observations of E D W Greig (1912) on this point are of special interest Greig states that during the Jaggannath Fair at Puri in 1912 when cholera was raging in the town he 'examined a number of flies taken near collections of cholera cases and found that they were harbouring the cholera vibrio on their external appendages and also in their alimentary tract' W C Ross (1928), in reference apparently to this observation, states that Greig found that 75 per cent of the flies caught in the centre of the city, in the neighbourhood of the temple, were heavily infected with cholera, both externally and internally, whilst those caught a mile or more away from the temple were infected only to a small extent, and flies caught at a greater distance were not infected More recently Dunn and Sarangam Khan (1929) examined 152 batches of 10 flies each caught at Hardwar during a cholera epidemic, and isolated non-agglutinating vibrios from 35 per cent of batches obtained from 76 different localities They isolated vibrios from peptone cultures in about the same number of instances in which the flies were washed and crushed, respectively, from which they inferred that probably the vibrios were on the surface of the body of the flies and not in the internal organs These observations establish nothing more than that flies in nature are capable of harbouring cholera-like vibrios and that they are capable, in consequence, of acting as passive agents in spreading the disease.

For a considerable time the observations made during the course of this investigation were in agreement with the findings of previous workers and all

attempts to recover vibrios from flies more than 24 hours after feeding them on a cholera emulsion failed

In view of the uniformly negative results of feeding experiments beyond 48 hours, it was proposed to stop the experiments, but it was eventually decided, having regard to the strong epidemiological evidence incriminating the house-fly and to the work of Almquist, Enderlein, Mellon, and Hadley on the life-cycle of bacteria, to carry out experiments over longer periods and it is with the result of these experiments that this report is solely concerned

Experimental details

A preliminary examination of flies caught in latrines and markets made it evident that they belonged to a number of different species and from the analogy of early work on malaria it was recognized that if a host-parasite relationship was to be investigated the species of the fly employed in the experiments might be a matter of considerable importance The species of fly used in these experiments could not be confidently identified and it was therefore decided to work mostly with insects bred out from specimens caught at latrines (latrine flies) For breeding purposes the flies were caught in butterfly nets and particulars of each catch were noted Insects bearing large number of eggs were selected for breeding purpose The flies layed their eggs on horse-dung in small cages, one fly being placed in each cage The horse-dung was collected immediately it was dropped so that no flies were able to lay eggs on it before it was collected Sterilized dung was found to be less suitable for egg laying than the fresh dung After the eggs had been deposited and young larvæ had hatched out they were transferred to wide-mouth bottles with tight cotton-wool plugs so that there was no risk of the larvæ wandering out of the cage When the pupal stage was reached the plug was removed and the bottle was placed in a tall cage in which the adults were able to run about and to fly They were fed on sterilized meat and plain water Two or more flies from each batch were killed and preserved for identification When the insects were one day old they were taken out, one by one, in ordinary test-tubes for feeding on an emulsion of *V choleræ*

The cholera culture used in the experiments was originally received from Central Research Institute, Kasauli It was plated out on agar and a single typically smooth colony was picked up in Dunham's solution (pH of 7 8 or 8) The organism used throughout these experiments exhibited typical vibrionic morphology, was actively motile and agglutinated fully against a high titre cholera serum (also received from Central Research Institute, Kasauli) It was daily sub-cultured on agar slope A 24 hours' growth was emulsified in sterilized tap water and mixed with an equal amount of sterilized milk In feeding, carbohydrates were avoided, except natural lactose in milk, so as to minimize acid production in the gut of the fly The milk and cholera emulsion were well shaken in order to distribute the organisms thoroughly For feeding purposes the mouth of the test-tube containing the fly was closed with a thin

piece of cork through which a small hole had been drilled Through this hole a piece of thin capillary tube ½ inch to 1 inch long was inserted so that its inner end slightly projected into the tube The outer end was now dipped in the cholera-milk emulsion, which by capillary action, rose to the top of the capillary tube The test-tube was then inverted, so that the emulsion remained flush with the inner end of the capillary tube It usually happened that the fly at once inserted its proboscis into the capillary tube and fed readily, but sometimes it required to be coaxed by giving the tube a few jerks, and thus directing the fly towards the capillary tube The gradual emptying of the visible portion of the capillary tube was a sure indication that the fly had taken in a sufficient amount of the emulsion This simple technique also prevented unnecessary fouling of legs and wings and gross contamination of the emulsion from outside By using a graduated capillary tube it was possible to regulate with considerable accuracy the dose of organisms which it was desired to administer Another advantage of this procedure was that the precise time of feeding was known with certainty After feeding, the infected flies were transferred to a big cage The same technique was also employed in determining whether the cholera vibrio could be inoculated, through the proboscis, into sterilized milk by infected flies

The examination of flies

For the recovery of organisms from infected flies, examinations were generally made at 2 hours, 24 hours, 2 days, 3 days, 4 days, 5 days, etc, after feeding them on the cholera-milk emulsion

The routine procedure adopted was to examine the following material at the times stated above with a view to the recovery of the cholera vibrio —

1 Piece of sterilized meat on which the infected flies had been sitting for a few minutes (food)
2 Sterilized milk on which the fly was fed through the capillary tube above described (fed milk)
3 Fresh fæcal deposits on the sides of a sterilized tube transferred to the culture medium by means of a platinum wire (fæces)
4 Whole fly chloroformed and thoroughly crushed in the culture medium (Cr)
5 Crop and gut The appendages of the fly were removed and the body was passed through a flame several times It was then dissected with aseptic precautions, and the crop and gut were transferred to culture medium (C and G)
6 A fly was killed and prepared into a block for sectioning by a double-embedding process

For cultures the following procedure was adopted —

1 The material to be examined was placed in a tube of Dunham's solution marked P_1 and incubated for 24 hours

2 A loopful from the surface growth of P_1 was transferred to another tube of Dunham's solution marked P_2 and incubated for 24 hours

3 From P_1 and P_2 plates of Esch's medium were streaked and marked E_1 and E_2 respectively

4 After 24 to 48 hours' incubation E_1 was examined and all the different types of colonies were marked out and numbered Smears were made and stained with dilute carbol fuchsin and examined All suspicious colonies were picked up in Dunham's solution and after 24 hours' incubation they were streaked on agar slopes and incubated They were kept in cold storage till required for further study If E_1 failed to reveal the presence of typical vibrios, E_2 plates were similarly examined

5 Pure growths on agar slopes showing even the slightest vibrionic morphology were further studied as to their cultural, biochemical and serological reactions

Considerable difficulty was experienced in keeping laboratory-bred flies alive in captivity, and the mortality became increasingly heavy as winter approached

In the first five experiments the flies were kept at laboratory temperature and no attempt was made to control accurately the temperature and humidity conditions, except that, with the onset of cold season, they were placed at night in a wooden box covered with a wet towel and during the day the cages were placed in a verandah, partly in the sun and partly in the shade In the sixth experiment the fed flies were kept most of the time in an incubator at 83°F dry bulb and 74°F wet bulb temperatures

The results of the experiments are tabulated below and the temperature conditions, as recorded in the laboratory, are noted against each —

With minor variations all the vibrios, including those isolated 4 days and 5 days after feeding resembled the original vibrio in character, viz , produced on agar plate, in 24 hours, circular, lenticular translucent colonies of light cream colour about 2 to 3 mm in diameter with smooth shining surface and homogeneous structure The colonies, which exhibited entire or slightly crenated edges, were moderately raised, undifferentiated or with a small knob in the centre, and were easily emulsifiable In peptone they gave a moderate uniform turbidity and usually a slight powdery deposit which could be easily dispersed by shaking No surface pellicle was observed except in the case of the vibrio isolated from food infected by flies four days after feeding on cholera-milk emulsion in which there was a complete thick surface pellicle It may be added that on agar slope this organism exhibited a more transparent and shining growth than the original organism

The cultural reactions of the vibrios in this experiment were like the typical vibrio except that the stroke on agar slope gave a less raised but more shining

EXPERIMENT No 1
August 25–29, 1930

Time after feeding flies on cholera-milk emulsion	CROP AND GUT			FOOD			CRUSHED FLY			Temperature conditions
	Morphologically typical vibrios	Cholera-red reaction in P_1 or P_2	Agglutination	Morphologically typical vibrios	Cholera-red reaction in P_1 or P_2	Agglutination	Morphologically typical vibrios	Cholera-red reaction in P_1 or P	Agglutination	
2 hours	+	+		+	+	+	—	—		Average maximum 90·5°F Average minimum 81·1°F Average at 8 A.M. 81·6°F
24 "	—	—		+	+	+	+	—		
48 "	—	+		—	+		—	+		
3 days	—	+		—	+		—	—		
4 "	—	—		—	+		—	+		

Experiment No 2

31st August to 10th September, 1930

Time after feeding flies on cholera-milk emulsion	Crop and gut			Food			Crushed fly			Temperature conditions
	Morphologically typical vibrios	Cholera-red reaction in P_1 or P_2	Agglutination	Morphologically typical vibrios	Cholera-red reaction in P_1 or P_2	Agglutination	Morphologically typical vibrios	Cholera-red reaction in P_1 or P_2	Agglutination	
2 hours	+	+	+	+	+	+	+	+	+	Average maximum 56·7°F Average minimum 81·9°F Average at 8 A.M. 82·3°F
24 „	—	+		+	+	+	—	+		
2 days	—	+		—	+		—	+		
3 „	—	+		—	+		—	+		
4 „	+	+	+	+	+	+	+	+	+	
5 „	+	+	+	+	+	+	+	+	+	
6 „	—	+		—	+		—	—		
7 „				—	+					
8 „				—	+					
9 „				—	+		—	+		
10 „							—	—		

EXPERIMENT No 3

October 3–8, 1930

Time after feeding flies on cholera-milk emulsion	Crop and gut			Food			Crushed fly			Temperature conditions
	Morphologically typical vibrios	Cholera-red reaction in P_1 or P_2	Agglutination	Morphologically typical vibrios	Cholera-red reaction in P_1 or P_2	Agglutination	Morphologically typical vibrios	Cholera-red reaction in P_1 or P_2	Agglutination	
2 hours	+	+	+	+	+	+	+	+	+	Average maximum 81·3°F Average minimum 81·1°F Average at 8 A.M. 81·1°F
8 „	+	+	+	+	+	+	+	+	+	
24 „	—	—		—	+ D		—	+		
32 „	—	+		—	+ D		—	+		
48 „	—	—		—	—		—	—		
54 „	—	—		—	+		—	—		
70 „	—	—		—	—		—	—		
76 „	—	—		—	—		—	—		
92 „	—	—		—	+		—	—		
98 „	—	—		—	+		—	—		
112 „	—	—		—	—		—	—		

EXPERIMENT No 4

October 4–9, 1930

Time after feeding flies on cholera-milk emulsion	Crop and gut			Food			Crushed fly			Temperature conditions
	Morphologically typical vibrios	Cholera-red reaction in P_1 or P_2	Agglutination	Morphologically typical vibrios	Cholera-red reaction in P_1 or P_2	Agglutination	Morphologically typical vibrios	Cholera-red reaction in P_1 or P_2	Agglutination	
2 hours	+	+	+	+	+	+	+	+	+	Average maximum 85·8°F Average minimum 82·1°F Average at 8 A.M. 82·1°F
8 „	+	+	+	+	+	+	+	+	+	
24 „	—	—		—	—		—	+ D		
32 „	—	—		—	+		—	+ D		
48 „	—	—		—	—		—	—		
54 „	—	+		—	+		—	+		
70 „	—	+ D		—	+ D		—	+ D		
76 „	—	+ D		—	+ D		—	+ D		
92 „	—	+ D		—	+ D		—	+ D		
98 „	—	+ D		—	+ D		—	+ D		
112 „	—	—		—	+ D		—	+		
118 „	+	+	+		+ D		—	+ D		

EXPERIMENT No 5

October 20–25, 1930

Time after feeding flies on cholera-milk emulsion	Crop and gut			Food			Fed milk			Faeces			Crushed fly			Temperature conditions
	Morphologically typical vibrios	Cholera-red reaction in P_1 or P_2	Agglutination	Morphologically typical vibrios	Cholera-red reaction in P_1 or P_2	Agglutination	Morphologically typical vibrios	Cholera-red reaction in P_1 or P_2	Agglutination	Morphologically typical vibrios	Cholera-red reaction in P_1 or P_2	Agglutination	Morphologically typical vibrios	Cholera-red reaction in P_1 or P_2	Agglutination	Average maximum 82·3°F Average minimum 75·8°F Average at 8 A.M. 75·8°F
2 hours																
24	+	+	+	+	+	+	+	+	+		+					
32 ,,																
48 ,,																
3 days										+	+					
4 ,,				+	+		+	+		+	+		+			
5 ,,				+	+	+	+	+		+	+	+	+			

EXPERIMENT No 6

This experiment was conducted as before, but in order to avoid any possibility of re-infection, no food or water was put in the cage and each fly was fed daily on sterilized milk by the capillary tube technique The cages were also disinfected by exposure to the sun and changed daily

Time after feeding	Crop and gut			Food			Fed milk			Faeces			Crushed fly		Temperature conditions
	Morphologically typical vibrios	Cholera-red reaction in P_1 or P_2	Agglutination	Morphologically typical vibrios	Cholera-red reaction in P_1 or P_2	Agglutination	Morphologically typical vibrios	Cholera-red reaction in P_1 or P	Agglutination	Morphologically typical vibrios	Cholera-red reaction in P_1 or P_2	Agglutination	Morphologically typical vibrios	Cholera-red reaction in P_1 or P_2	Agglutination	
2 hours	+		+	+		+	+	+	+	+		+		+	+	In the laboratory room Average maximum 74·9°F Average minimum 71·3°F Average at 8 hours 71·5°F The flies were, however, kept most of the time in an incubator at 83°F dry bulb and 74°F wet bulb temperature
24 "							+	+								
Over 24 hours																
2 days																
3 "																
4 "																
Over 4 days																
5 days	Atypical															
6 "																

Not done

Not done

and transparent growth than others The colonies were also somewhat smaller in size

Colonies on agar plate of the vibrios isolated from 5 days' fæces differed from those of the typical vibrios in that they were smaller in size, less raised, gummy in consistency and more shining In peptone the usual uniformly turbid growth without surface pellicle was obtained but it was less profuse On agar slope the growth was thin and glossy

As a general rule in these experiments, recently isolated vibrios from the flies 4 or 5 days after feeding were morphologically distinguishable from the original culture of those isolated from the insects 2 hours or 24 hours after feeding, in that they were thinner, more variable in size and shape, had more open curvature and presented a whip-like appearance at the end Two or more vibrios joined on ends were more frequently met with, suggesting a more rapid multiplication

The flies used in these experiments have been sent to an expert entomologist for identification The examination of sections and of some of the organisms isolated during the course of this inquiry is still in progress, and it is proposed to deal with this material in subsequent communications

Summary

The experiments recorded above are admittedly meagre, and it is not at present claimed that they justify the conclusion that a true host-parasite relationship exists between the fly and the vibrio It would, however, seem in the first place that the vibrios are capable of surviving in the fly for a period of at least five days Secondly, it would appear that the cholera vibrio apparently disappears from the body of the fly after 24 hours or so, but that it re-appears on or about the fifth day, at which time the fly is capable of infecting food by its fæces Thirdly, it has been shown that infection of milk via the proboscis can take place up to 24 hours, but it has not yet been proved that infection via the proboscis can take place on and after the fifth day

It would be premature at present to discuss the significance of these observations, but they seem to suggest that possibly one phase of the life-cycle of the cholera vibrio is passed in the body of the house-fly and that this insect may play a more important part in the transmission of cholera than has hitherto been suspected

V Conclusion

It is not proposed to discuss further the epidemiological and experimental grounds which have led to the tentative conclusion that the house-fly plays an important part in the transmission of cholera, except to state it is recognized that the experimental evidence pointing to the accuracy of the hypothesis is meagre and that further investigation, both in the field and in the laboratory, is necessary before the theory can be regarded as embodying an

established fact It would ordinarily be premature, if not improper, to put forward tentative conclusions based upon an admittedly incomplete inquiry, but several reasons appear to justify a departure from this sound rule in the present instance

In the first place the results of the epidemiological investigation, apart altogether from any inferences drawn therefrom, appear to be of sufficient interest and importance to warrant their publication, secondly, the investigation appears to open up a field of inquiry which calls for exploration from the standpoint of the bacteriologist, the entomologist as well as the experimental epidemiologist, and, lastly, with the onset of the cold weather the experimental part of the investigation has had to be temporarily suspended owing to the difficulty of keeping house-flies alive under laboratory conditions for the period necessary for experimental purposes

The most striking result of the investigation is undoubtedly the suggestion that cholera may perhaps have to be numbered amongst the insect-borne diseases It would indeed seem that just as the plague bacillus is sometimes transmitted through the medium of air (pneumonic plague) and sometimes, more especially in hot climates, by the agency of rat-fleas (bubonic plague), so the cholera vibrio may perhaps be sometimes disseminated through the medium of water and sometimes, more especially in tropical countries, by the agency of house-flies It would moreover appear that in hot countries insect transmission is of predominant importance and that the strikingly dramatic outbreaks associated with the massive pollution of water are relatively rare incidents, if not accidents, in the natural history of the disease It is a far cry to the air-borne theory so vehemently advocated by Bryden and Cunningham but it would seem that this theory may not, after all, have been so wide of the mark, and that there is something to be said for evacuating troops from an infected locality by marching them across the prevailing wind, since this procedure is at least calculated to remove them to an area where the fly population is not infected

May it not be that the air-borne theory and the water-borne theory are both half-truths and that cholera is both an air-borne (or fly-borne) and a water-borne disease? May it not be also that the undue stress laid upon water-borne transmission is largely responsible for the small progress made in controlling the disease? These speculations are merely mentioned to indicate the trend of thought engendered by the present hypothesis, but before concluding this preliminary report it may be well to consider the practical implications arising out of the observations recorded in the foregoing pages

In the first place it is now tolerably clear why the measure of success attending existing methods of combating cholera has been disappointingly small

Explosive epidemics which appear, indubitably, to be due to the massive pollution of drinking water are, it has been shown, of infrequent occurrence They can by their very nature be neither foreseen nor controlled, and they

can only be prevented by arrangements that will eliminate the possibility of drinking water being inoculated with the cholera vibrio

It is also clear that these measures, which imply in the case of towns, the provision of a piped supply of filtered water, are not alone capable of exercising any definitive influence upon the protracted epidemics which is the usual type of epidemic in urban areas In these epidemics, it is surmised, the main agent in spreading the disease is the house-fly, but even if this hypothesis be eventually proved to be untenable, it does not seem to be open to doubt that bad sanitation, and more especially bad conservancy, is a factor of the greatest importance in determining their occurrence In these circumstances it is no longer a matter of surprise that the provision of piped water-supplies and the rigorous disinfection of the drinking water, combined with other routine measures, in the absence of measures designed to eliminate the gravely insanitary conditions which almost universally prevail in Indian towns, have proved of little value The experience gained at Thanesar renders it also clear that even under the most favourable conditions it is not possible to prevent the entry of acute 'carriers' into uninfected areas It is furthermore clear that medical inspection posts are of little value in this respect and it also follows that the mass inoculation of pilgrims, even if it were practicable, would not constitute an effective method of preventing the spread of cholera by pilgrims, unless, indeed it is proved that the acute 'carrier' is rendered harmless as the result of inoculation

What then is the practical lesson to be derived from this investigation? It has been shown that more than half the outbreaks of cholera are limited to a few seizures and that it is mainly in towns where conservancy arrangements are peculiarly defective that importation occasions, after an interval, a protracted epidemic In these circumstances it would appear that one of the most important, if not the most important, method of controlling cholera is the provision of an efficient system of sanitary control, more especially in connection with the collection, removal and disposal of night-soil and refuse

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TWO INTESTINAL MASTIGOPHORA FROM AN INDIAN BULL

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THROUGH the kindness of Mr H Cooper, M R C V S, Indian Veterinary Service, of the Imperial Institute of Veterinary Research, Muktesar, we received on the 25th of August, 1930, two sealed tubes of normal saline containing intestinal flagellate protozoa from the fæces of a bull (*Bos taurus*) of the Himalayan foot-hills breed

The history of this animal was as follows It passed through a severe attack of rinderpest, which caused a relapse of the coccidiosis from which it had previously suffered, and died from the combined effects of rinderpest and coccidial dysentery on the 4th August, 1930 The fæces, when examined for coccidial oocysts on the 1st August, showed the presence of flagellate organisms, about one per microscope field The sample was centrifuged and portions of the deposit inoculated into tubes containing 10 c c of normal saline, these were incubated at 22°C On the fourth day large numbers of flagellate organisms were present, and sub-inoculations were made into further tubes of normal saline These again showed a fairly abundant growth of flagellate organisms on the fourth day A third set of sub-cultures again showed abundant growth on the fourth day, and two of these tubes were despatched to Calcutta The parasites in the original tubes died out by the seventeenth day

On receipt of the two tubes in Calcutta, the material was examined in the fresh state under dark-ground illumination, and it was found that two different intestinal flagellate protozoa were present one a small flagellate with

two flagella and a very conspicuous cytosome, the other a much larger one, which gave the appearance of having an undulating membrane, although this was a little uncertain (Mr P R Krishna Iyer of the Muktesar Institute, who examined the fresh specimen of fæces from which the original cultures were taken states that the presence of an undulating membrane and of an axostyle were distinctly seen)

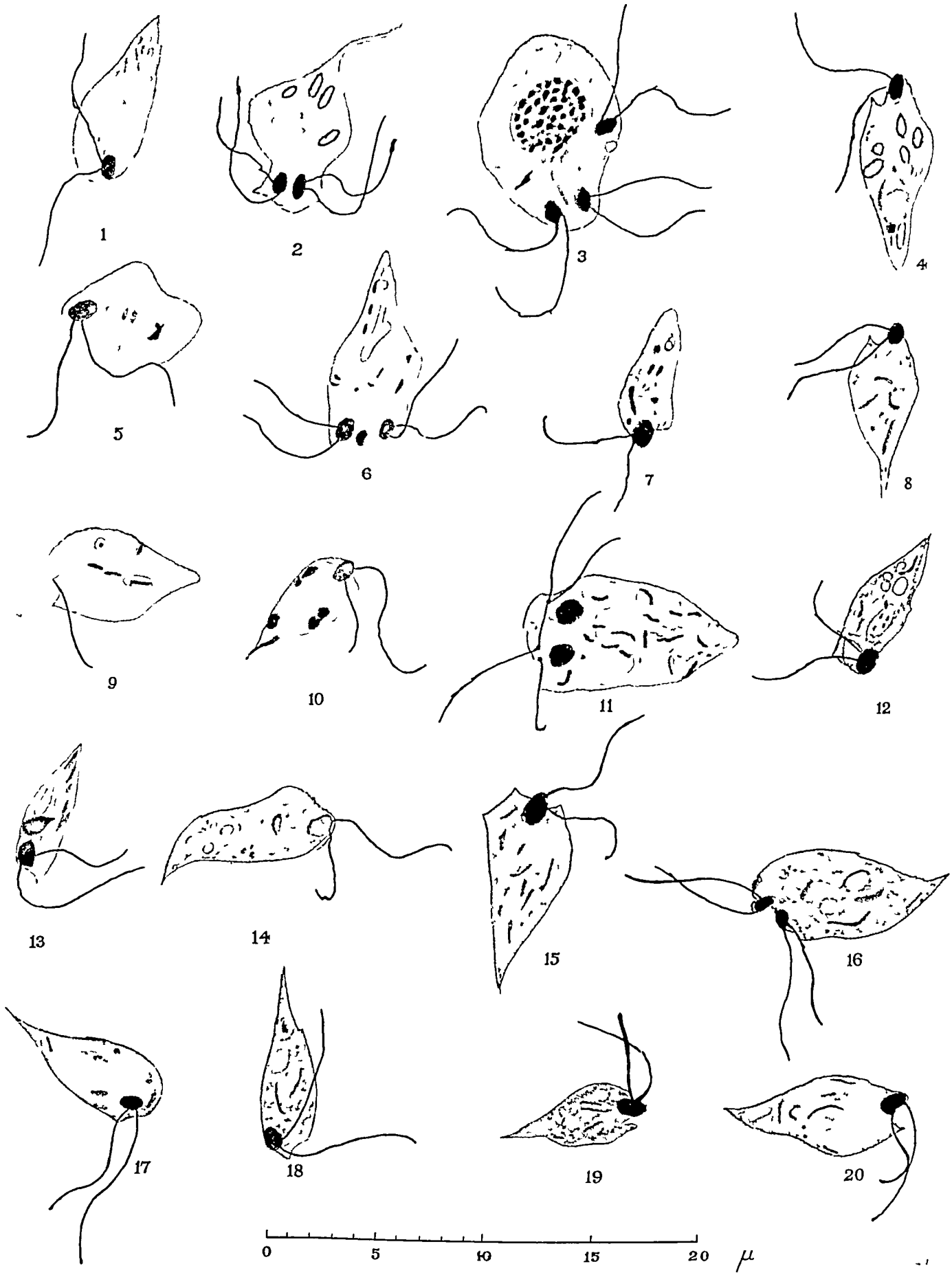
Fresh sub-cultures were now taken in Row's hæmoglobin saline medium (Row, 1914), and in the HSre medium recommended for cultivation of *Entamœba histolytica* by Dobell and Laidlaw (1926), which we have found to be an excellent medium for the cultivation of intestinal Mastigophora These were incubated at 37°C for 3 days, but showed no growth On the third day very small pear-shaped bodies were seen, which were not motile (cysts of the Embadomonas about to be described) These were present only in scanty numbers

Fresh sub-cultures from the original tubes received from Muktesar were now taken in Row's medium and in Dobell and Laidlaw's medium, and some were incubated at 22°C, and others at room temperature (21° to 35°C) These showed a rich growth of the smaller flagellate by the third day in the tubes at 22°C, in the cultures kept at room temperature growth also took place, but more slowly Encystment also occurred in several of the tubes kept at 22°C In cultures placed in the ice chest (13° to 18°C) all flagellates died out within 24 hours

The larger flagellate protozoon unfortunately died out in the cultures, and our material for the study of this organism has been very scanty The smaller organism–an Embadomonas–has been maintained in sub-cultures at 22°C up to the time of writing, a period of more than six weeks In a culture more than a month old only cysts were seen, sub-cultures from this culture were taken in Dobell and Laidlaw's medium, and incubated at 22°C, these showed numerous motile Embadomonas at the third day

In order to obtain further material for the study of these organisms, cultures were taken in Dobell and Laidlaw's medium from the freshly passed fæces of six cows at the Keoriapukri Kala-azar Treatment Centre near Calcutta Of these two gave a rich growth of a coprozoic Bodo, one showed very scanty ciliate protozoa present, and the remaining three showed no protozoa present Neither of the two flagellate protozoa originally encountered in the cultures from Muktesar were present

In the meantime the senior author had written to Mr Cooper, asking for further material Mr Krishna Iyer kindly inoculated seven tubes of Row's medium from the fæces of seven hill bulls of the same breed, in three specimens of which he had again found the smaller flagellate organism These seven bulls were all suffering from diarrhœa at the time when the cultures were taken On receipt of the cultures at Calcutta, sub-cultures were taken, and all seven sub-cultures were found to contain a rich growth of the same Embadomonas as before



Embadomonas ruminantium, motile forms Fixation with osmic acid, followed by methyl alcohol, Giemsa's stain

We may next proceed to describe the two organisms found in the original cultures As will be seen, we had abundant material for the study of the Embadomonas, but only scanty material for the study of the larger flagellate organism

Embadomonas ruminantium n sp

The motile forms of the smaller flagellate are illustrated in Plate LXIX Mackinnon (1915), who first described the genus *Embadomonas* in 1911, gives the following definition of this genus —

'This genus contains small slipper-shaped flagellates, characterized by a very large cytostome bordered by prominent lips, which are more or less siderophilous, and two flagella, not so long as the body, one acting as an organ of locomotion, and the other lying in the cytostome, the spherical nucleus is placed at the anterior end of the body, the two basal granules, from which arise the two flagella, lie at the anterior border of the cytostome There is a definite periplast, which prevents deformation of the body The anterior part of the body shows a well-marked torsion The cysts are relatively small, and are ovoid in form'

It will be seen from Plate LXIX that the smaller flagellate from the Muktesar bulls conforms in every way to this description The cytostome at the anterior end is very prominent The motile forms vary in length from 6 5 μ to 9 μ, and in greatest breadth from 2 μ to 4 μ The posterior end is generally tapered, but there is no axostyle Of the two flagella, the anterior one is longer and thinner than the intracytostomic one (Plate LXIX, fig 20) As seen under the dark ground, the anterior flagellum is very active, as it lashes, the tip of the flagellum tends to hook down towards the cytostome, with the result that forward movement takes place in a jerky manner The movement of the intracytostomic flagellum is slower, more continuous, almost rippling in character Yeasts and bacteria are freely ingested, but when rice starch was added to the medium, no starch particles were ingested Larger and more spherical forms were encountered, but in all instances these proved to be dividing individuals (Plate LXIX, figs 3, 11 and 16) A peculiar feature of some of the cultures was the presence of somatella with 3, 4, 5–in one instance as many as 8–nuclei, and double this number of flagella, these appeared to be multiple fission forms, but it is difficult to say whether this is a normal process, and not the result of the abnormal environment of culture Division is usually by binary longitudinal fission (Plate LXIX, figs 2, 6, 11 and 16) In old cultures many of the flagellates become parasitized by Sphærita (Plate LXIX, fig 3), in the fresh state the Sphærita colony inside the flagellate shows up as a greenish, refractile, morula-like mass Two and even three colonies of Sphærita may be encountered within the same flagellate The cytoplasm of the animal is frequently much vacuolated, especially towards its posterior end

With regard to culture media, we have used the following —

(*i*) Dobell and Laidlaw's HSre medium

(*ii*) Row's hæmoglobin saline medium

(*iii*) The medium used by Barret and Yarborough (1921) for the cultivation of *Balantidium coli*

(*iv*) The medium used by Noguchi (1917) for the cultivation of leptospira

(*v*) Hay infusion

The flagellates grow best in (*i*), there is a fair growth in (*ii*), a very scanty growth in (*iii*), and none at all in (*iv*) and (*v*) The best growth is obtained at 22°C , slower growth occurs at room temperature (21° to 35°C), growth is inhibited at 37°C and in the ice chest at 13° to 18°C It may seem strange that an intestinal parasite of a warm-blooded host should not grow at 37°C , and should grow well at 22°C , but we attribute this to the heavy bacterial contamination at the higher temperature killing off the flagellates, this would also explain why better cultures were obtained at 22°C than at room temperature of 21° to 35°C Hogue (1921) obtained successful cultures of *Embadomonas intestinalis* of man at 35°C , whilst Wenyon (1921) also obtained cultures of species of Embadomonas from the guinea-pig, rat, tortoise and frog at 24°C and at 30°C Seeing that the same species of Embadomonas was obtained in cultures from eight consecutive bulls examined, the organism cannot be other than a true parasite of the species of bull concerned Although these animals were all suffering from either diarrhœa or dysentery, there is no evidence that the Embadomonas concerned had any causative connection with the intestinal disturbance *

Cysts—Plate LXX illustrates the cysts, as seen in films from cultures fixed with Schaudinn's fixative and stained by Heidenhain's iron-hæmatoxylin stain The cysts are very small, 3 to 4 5 μ in length and slightly less than this in breadth They are usually ovoid in shape, sometimes pyriform

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* Since the present paper was written, we have had further information from Mr Krishna Iver, who writes as follows —

'The only culture media employed in the cultivation of the organisms were Boyd's serum saline medium and Row's hæmoglobin saline medium, both incubated only at 22°C, and not at 37°C Trichomonas seemed to thrive better in the former and they were found to be actively motile and multiplying for a long time in the same tube in this medium for over one month

Row's hæmoglobin saline medium was found to be decidedly superior to Boyd's for the cultivation of Embadomonas The Trichomonas organisms, however, could not adapt themselves to this and all of them perished during the course of the second sub-inoculation leaving only a pure culture of Embadomonas behind Due to the very active multiplication of secondary organisms in Row's hæmoglobin saline medium, sub-cultures had to be made every 6 days and I have at present a pure culture of Embadomonas going in this medium

It may be of interest to mention here that since forwarding the Embadomonas and Trichomonas material to Colonel Knowles, I have had the opportunity of examining several other samples of fæces derived from rinderpest animals that had developed diarrhœa, and on microscopical examination, almost all of these samples revealed the presence of both Trichomonas and Embadomonas, with or without the presence of coccidia'

PLATE LXX

Embadomonas ruminantium, cysts Fixation with Schaudinn's fluid Heidenhain's iron-hæmatoxylin stain

Owing to their extremely small size it is difficult to make out details of the structures within the cyst, even in a well-differentiated film In iodine preparations the cysts are seen to be mononucleate, and the 'shadow outline' of the cytostome stands out clearly In films fixed by osmic acid and stained by Giemsa's stain the nucleus is seen to consist of an achromatinic nuclear membrane with no chromatin on it, and a central small karyosome

Plate LXX has been drawn to show the exact appearances seen within the cyst It is very hard to interpret these appearances The single looped line, or the double lines, more or less parallel, and usually with a deeply staining portion between them, appear to be the remains of the cytostome or of its thickened margins The elongated or dumb-bell shaped nucleus described in the cysts of *Embadomonas intestinalis* by Wenyon and O'Connor (1917) was not seen in the present species Mackinnon (1915), for the cysts of *Embadomonas alexeieffi* of the crane fly, describes and figures disintegration of the nuclear membrane and the chromatin as escaping in groups of granules into the cytoplasm of the cyst In the present species there are certainly present very minute and deeply staining granules within the cyst, but they appear to consist of volutin rather than to be derived by disintegration of the chromatin of the nucleus

A very characteristic feature of the cyst of the present species is the shrinkage of the contents away from the wall of the cyst, this, however, may be due to the fixative used, cold Schaudinn's fluid This appearance is not seen in cysts in films fixed with osmic acid and stained by Giemsa's stain (The figures given for size are from films fixed with Schaudinn's fixative and stained by iron-hæmatoxylin) The cyst wall appears to be thinner than that of *E alexeieffi*

Discussion—The species of Embadomonas previously described are as follows —

E agilis Mackinnon, 1911, in tipulid and trichopteran larvæ This varies in size from 4 by 1 5 μ up to 11 by 3 μ, the cysts measure 3 5 by 3 μ to 4 by 3 μ

E alexeieffi Mackinnon, 1911, in tipulid larvæ It measured 7 to 16 μ by 5 to 9 μ, whilst the cysts were 5 to 6 μ by 4 to 5 μ

E intestinalis (Wenyon and O'Connor, 1917) In the living condition the flagellates measured from 4 to 9 μ in length by 3 to 4 μ in breadth, the cysts were from 4 5 to 6 or even 7 μ in length, by 3 to 4 5 μ in breadth

E wenyoni (Fonseca, 1917) from the Brazilian monkey *Cebus carya* Wenyon (1926, p 620) considers that this form closely resembles *E intestinalis* of man, 'with which it may be identical'

E sinensis Faust and Wassell, 1921, seen in diarrhœic fæces from nine patients in China Wenyon (1926, p 619) considers that 'it is exceedingly doubtful if *E sinensis* is a distinct species from *E intestinalis*, especially as the encysted forms are alike'

E belostomæ (Brug, 1922) from the water bug *Belostoma* sp in Java Wenyon considers that 'it actually shows no specific difference from other species which have been described'

E cuniculi Collier and Boeck (1926) from the cæcum of the rabbit In the living and motile state this measures from 7 5 to 13 μ in length by 5 5 to 9 5 μ in breadth

E bradypi Hegner and Schumaker (1928), from the three-toed sloth *Bradypus griseus griseus* This has a length of from 4 3 to 5 5 μ, with a breadth of 3 6 to 4 4 μ The cyst is characterized by having a deeply staining pad at the anterior end which may be a swelling of the cyst wall

E caviæ Hegner and Schumaker (1928) from the guinea-pig–presumably the same species as that cultivated from the guinea-pig by Wenyon (1921) but not named by him This is a very small species, with a length of from 3 4 to 5 2 μ, and a breadth of from 3 to 4 μ

E ovis Hegner and Schumaker (1928) from the sheep This has a length of from 4 to 6 μ, and a breadth of from 3 1 to 4 4 μ

In addition to these, flagellates of the genus *Embadomonas* have been cultivated by Wenyon (1921, 1926) from the intestinal contents of the guinea-pig, the rat, a tortoise and a frog, but have not been specifically named da Fonseca and Muniz (1926) have also described an Embadomonas-like form from the cockroach These authors, however, maintain that the genus *Waskia* differs from the genus *Embadomonas*, they consider that the parasite of the cockroach belongs to the genus *Embadomonas*, but that the generic name *Waskia* should be retained for the form found in man

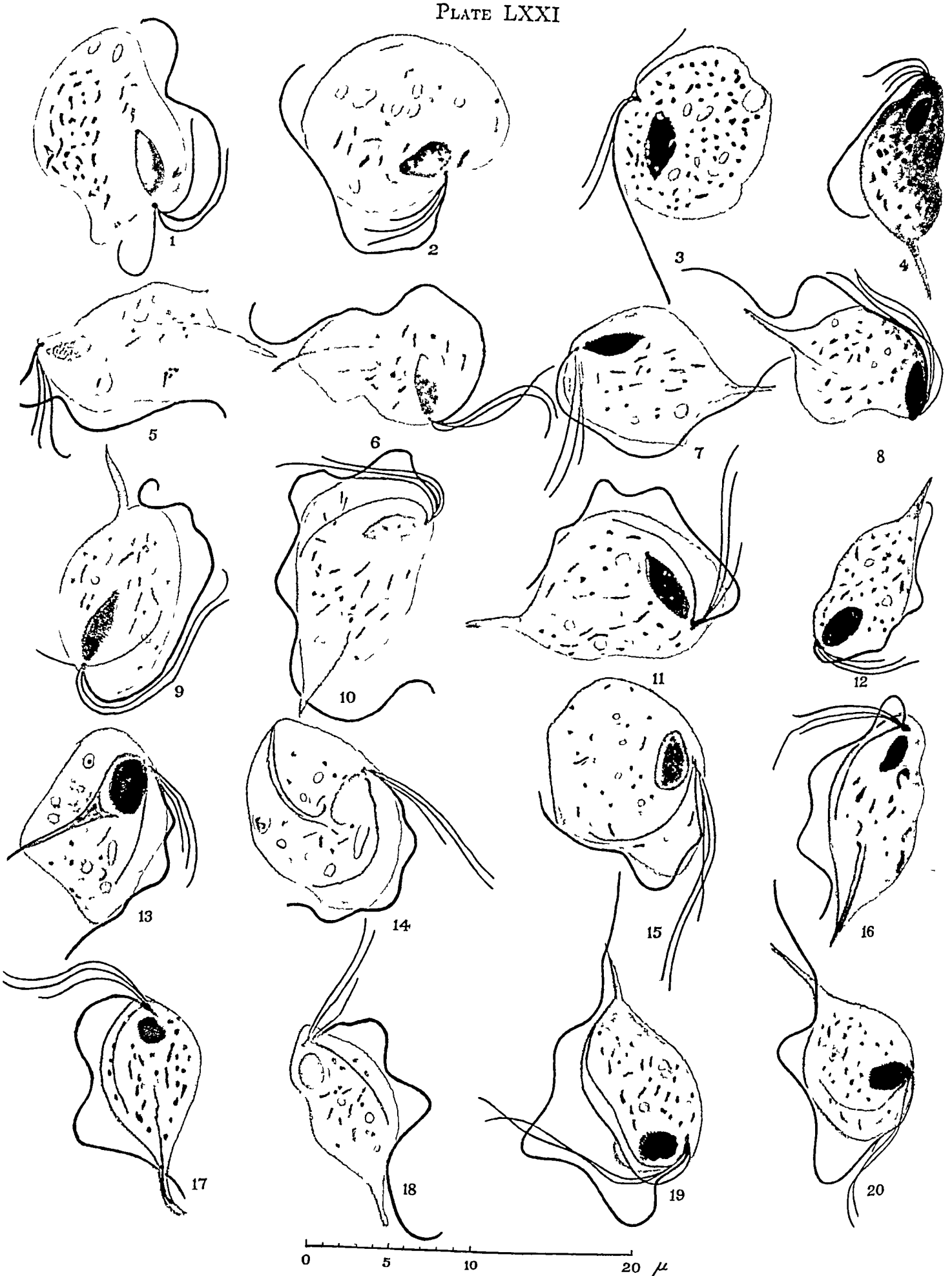
Of these different species, the one which comes closest to the Embadomonas of the bull is *E agilis* There seems to be some difference of opinion amongst zoologists as to whether, when two parasites which are morphologically so similar that they cannot be differentiated from one another occur in two widely separated types of hosts, they should be put into one and the same or into two different species Where the hosts are zoologically very closely related, the parasites in all probability belong to the same species, thus Dobell (1928) has shown that *Entamœba histolytica* of man and of Macacus monkeys are one and the same parasite Other authors tend to create new species on very little evidence

The bull, however, is so widely separated zoologically from tipulid and trichopteran larvæ, that we consider that the Embadomonas of the former should receive a specific name of its own We accordingly suggest the name *Embadomonas ruminantium* n sp

Trichomonas ruminantium Braune, 1913

As already stated, the second, larger flagellate soon died out in the cultures, and hence our material for the study of it was but scanty Plate LXXI illustrates the forms seen No cyst was encountered at any time

PLATE LXXI



Trichomonas ruminantium, motile forms Fixation with osmic acid, followed by methyl alcohol, Giemsa's stain

The body is pyriform but very liable to distortion It varies in length from 11 to 16 μ, and in greatest breadth from 5 to 10 μ (in fixed and stained specimens) There is a large oval nucleus near the anterior pole From a group of basal granules just in front of the nucleus three delicate anterior flagella arise, these beat together with a sweeping action, one of them appears to be much shorter than the other two (Plate LXXI, figs 2, 9, 11, 12, 13, 14, 15, 17, 18 and 19) From the same group of basal granules arises a posterior, trailing flagellum, this is definitely thicker than the flagella of the anterior group As it passes backwards along the body of the animal it shows a special tendency to adhere to the lateral margin, as is exemplified throughout Plate LXXI It finally projects free behind the posterior pole (Plate LXXI, figs 6, 7, 8, 9, 10, 13, 17, 18, 19 and 20)

The two most variable organelles in the animal are the axostyle and what may be termed the 'crescentic fibril', corresponding to the basal fibril in the undulating membrane of a Trichomonas The axostyle is sometimes absent (Plate LXXI, figs 1, 2, 3 and 15), but these are probably degenerating forms, sometimes, however, it is conspicuous (Plate LXXI, figs 4, 5, 6, 8, 10, 11, 13, 14, 16, 17 and 18) The crescentic fibril is sometimes inconspicuous or even apparently absent (Plate LXXI, figs 1, 2, 3, 4, 5, 6, 7 and 12), sometimes, however, it is very prominent (Plate LXXI, figs 9, 10, 11, 13, 14, 15, 16, 17, 18, 19 and 20)

It is clear from its morphology that this organism belongs either to the genus *Trichomonas* or to the genus *Eutrichomastix*, and it is very difficult to come to a decision on this point Braune (1913) has described from the rumen of cattle both a Trichomastix and a Trichomonas The former was about 8 μ in length with 3 anterior flagella, an axostyle, and an 'elastic fibril', he states however that he was not able to detect a trailing flagellum, and his single illustration recalls an Enteromonas rather than a Trichomastix The Trichomonas was about 8 μ in length, with 3 anterior flagella, a marked axostyle, often with a crescentic row of dots lying parallel to it, recalling the appearances encountered in *Trichomonas muris* He neither mentions nor figures an undulating membrane, but speaks only of a trailing flagellum (Schleppgeissel)

Presumably the form which we have encountered is one or other of these two species, and it remains to determine which This brings us to the very difficult question of the identity or otherwise of the genera *Eutrichomastix* and *Trichomonas* And here we cannot do better than quote a passage from Wenyon (1926, p 671), which summarizes this matter

'*Eutrichomastix* Kofoid and Swezy, 1915 This genus includes flagellates, which resemble *Trichomonas* except for the absence of an undulating membrane, the posterior flagellum of *Trichomonas* being represented by a trailing flagellum They have generally been known by the generic name *Trichomastix*, but owing to the fact that Vollenhoevan had previously proposed this name for an insect,

Kofoid and Swezy (1915) introduced the name *Eutrichomastix*. It seems probable that, in some cases at least, the *Eutrichomastix* forms are merely *Trichomonas* in which the posterior flagellum has become free. Chatton (1920) found that in cultures the *Trichomonas* of the guinea-pig might assume either form. Reichenow (1918, 1920) noted that occasionally in lizards the blood stream was invaded by *Eutrichomastix* from the intestine. In one case in which a lizard had died of such an infection, at the time of death the only forms present in the blood were of the *Eutrichomastix* type. On the next day, however, in addition to these there were other flagellates of the *Trichomonas* type present. Reichenow considers it possible that the latter had been derived from the former, and that the two types may be stages of one organism. In favour of this view is the well-known fact that where flagellates of the *Trichomonas* type occur, very frequently others of the *Eutrichomastix* form are present at the same time. Thus Dobell (1909) noted that *T. batrachorum* was often associated in the frog's intestine with *E. batrachorum*, and a similar association was noted by Prowazek (1904) in the case of lizards, and by Martin and Robertson (1911) in fowls. On the other hand, it appears that sometimes the flagellates are found in the *Eutrichomastix* form when *Trichomonas* is absent, as in the case of *E. serpentis* seen in a snake by Dobell (1907). The writer (Wenyon) has cultivated a *Trichomonas* of the tortoise (*Testudo radiata*), the python (*Python molurus*), and the frog, and in these cases there was no tendency for the flagellates to assume the *Eutrichomastix* form. For the present, therefore, it seems best to regard the flagellates as belonging to two distinct genera.

The flagellates of the genus *Eutrichomastix* have the same structure as those of the genus *Trichomonas*, except that all the flagella, which are four in number, are free, there being no undulating membrane. One of the four flagella usually functions as a trailing flagellum.'

Grasse (1926) believes that he has detected a polymorphic cycle of development in trichomonad flagellates, having shown that Trichomonas may assume the Eutrichomastix form, and vice versa, and having observed present together with the larger forms, small Trimitus forms with 2 or 3 flagella and an attached trailing flagellum. He supposes that Trimitus forms develop through a Eutrichomastix phase into a Trichomonas form, and that in some hosts Eutrichomastix remains as such without further development.

Such evidence as we have been able to collect in Calcutta all goes to show that *Trichomonas* and *Eutrichomastix* are two quite distinct genera. We have frequently cultivated species of Trichomonas from many different sources, and have never seen any Eutrichomastix forms in such cultures. In 1928 on examining the intestinal contents of a rat-snake (*Zamenis mucosum*) under the dark ground two flagellate protozoa of trichomonad type were present, one with, the other without, an undulating membrane. Cultures were taken in Row's hæmoglobin saline medium and in Dobell and Laidlaw's HSic medium

In stained films from the cultures three different species of flagellate protozoa could be identified with certainty, viz, a Trichomonas, a Tricercomonas, and a Eutrichomastix The Eutrichomastix later encysted, whereas the Trichomonas and the Tricercomonas did not By sub-culturing from the cysts only, a pure culture of Eutrichomastix in its motile phase was secured The organism was now carefully studied and compared with the Trichomonas of the same host By repeated sub-culture the Eutrichomastix strain was kept going from September 1928 to January 1929 On no occasion was any trace of an undulating membrane seen, and the flagellates remained true to morphological type

It is true that a dying Trichomonas may closely simulate a Eutrichomastix As pointed out by Dobell (1907) for *T serpentis,* as the animal degenerates the trailing flagellum tends to adhere to the margin of the body, whilst in the final phases amœboid forms are produced On the other hand in fresh material it is always possible, in our experience, to differentiate between the two, thus we have frequently studied *T lacertæ* and *E lacertæ* of the lizard In stained preparations there is no tendency in *E lacertæ* for the trailing flagellum to adhere to the margin of the body, whereas the undulating membrane of the Trichomonas is unmistakable Further, under the dark ground, the movements of the two animals are different As the movements of Trichomonas slow down, the rippling of the undulating membrane is unmistakable On the other hand, in Eutrichomastix the posterior, trailing flagellum stands out laterally away from the body, then lashes in against it, then stands out again, the movement recalling that of a railway signal arm

In the case of the species illustrated in Plate LXXI there is a very special tendency for the trailing flagellum to adhere to the margin of the body Further, Mr Krishna Iyer states that in the fresh material from which the cultures were taken, the presence of an undulating membrane was unmistakable

Lastly, there is the question of the 'crescentic fibril'

No such crescentic fibril is described or illustrated by Dobell (1907) for *E serpentis,* by Dobell (1909) for *E batrachorum,* by Mackinnon (1912) for Eutrichomastix of Tipula, by Braune (1913) for *E ruminantium,* by Reichenow (1920) for *E lacertæ* Martin and Robertson (1911) in *E gallinarum* show a faint chromatinic line, upon which lie a number of deeply staining dots of chromatin, but this lies parallel with the axostyle, and on the side away from the trailing flagellum

It is very hard to resist the conclusion that the crescentic fibril shown in Plate LXXI, figs 9, 10, 11, 13, 14, 15, 16, 17, 18, 19 and 20, is the strengthening basal fibril of an undulating membrane Accordingly we believe that the flagellate protozoon illustrated in Plate LXXI is *Trichomonas ruminantium,* though here studied in a degenerating phase The adherence of the trailing flagellum to the margin of the body represents an undulating membrane which

is in process of dissolution Figs 1, 2 and 3 appear to represent the dying pseudo-amœboid phases of the animal

* * * * * *

We cannot conclude this paper without expressing our most grateful thanks to Mr Cooper and to Mr Krishna Iyer for this most interesting material, and for their kindness in taking cultures for us

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STUDIES ON GOITRE PRODUCED BY CABBAGE

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THE discovery of Chesney, Clawson and Webster (1928) that cabbage, when it forms the main bulk of the food of rabbits, causes thyroid hyperplasia within the relatively short period of 15 to 30 days and considerable goitres after months of subsistence on the diet, has provided a ready and convenient means for the study of thyroid physiology and pathology The work of these observers has been confirmed and extended by Marine and his colleagues (1929, 1930) while Chesney and his co-workers (1929, 1930) have added considerably to their original discovery It is now definitely known that a *positive* goitrogenic agent exists in cabbage, that it is insoluble in water, unaffected by boiling, destroyed by drying in certain ways, and is more potent at certain ranges of pH (Marine *et al*, 1929, 1930) The goitre-producing potency of cabbage is said to vary with season, to be enhanced by steaming and to be inversely proportional to the ability of the cabbage to absorb iodine Steamed cabbage from which the juice has been expressed is almost as effective as the whole vegetable, while the expressed juice is only slightly effective (Marine *et al*, 1930) The goitre-producing action of cabbage is annulled by the simultaneous administration of iodine (Webster and Chesney, 1928) Goitres produced in this way are curable by iodine-therapy, their cure being associated with an increase in the metabolic rate (Chesney *et al*, 1928)

Purpose of the Investigation

The purpose of the present investigation was threefold to observe the goitre-producing action of cabbage, to study the changes in iodine-metabolism, as evidenced in the gland itself and in the urinary excretion of iodine, that might be brought about by the goitrogenic agent in cabbage, and, to discover substances antagonistic to this agent

Previous experience had shown that the first essential in an experimental investigation of this kind is to make sure of a perfectly normal universe of animals from which to begin observation In the rabbit the thyroid gland is amongst the most variable, both as to size and histological structure, of all organs of the body Brown, Pearse and v Allen (1925, 1926), in their statistical studies of organ weights in rabbits, found the co-efficient of variability in size of the thyroid gland to be as high as 70 3 in 349 healthy animals, and to range between 30 0 and 127 5 in 127 unhealthy animals, the thyroid thus exhibiting a variability greater even than that of the spleen, the corresponding co-efficients for which were 44 0 and 44 2 to 65 6 Apart from acute and chronic diseases which may cause the thyroid gland to vary either in the direction of increase or of decrease in size, statistical studies* in these laboratories have shown that the principal cause of the gland's variability in size is diet, further, this variability is a criterion of abnormality second only in importance to enlargement of the gland itself To ensure a wholly normal universe of experimental animals the stock diet should be so constituted that the size of the organ does not exceed certain limits at given periods of life while the co-efficient of variability in size remains low In Brown, Pearse and v Allen's (1926) extended series of 644 apparently normal rabbits the gross body-weight of the animals ranged between 1,400 and 3,500 grammes (median 2,225 grammes), while the weight of the thyroid ranged between 85 and 1,730 mg (median 200 mg), the co-efficient of variability in weight of the gland being 63 35 In the uniformly-fed, stock rabbits (from amongst which the animals used in this investigation were taken) the corresponding figures are range of body-weight, 1,450 to 2,440 grammes (median 1,880 mg), range of thyroid-weight, 129 to 428 mg (median 219 mg), co-efficient of variability in thyroid size, 34 2 While, therefore, the mean size of the thyroid gland in our own stock is slightly higher† than that in Brown, Pearse and v Allen's series, the co-efficient of variability is considerably less The stock diet in use for rabbits in these laboratories consists of cabbage, grass, carrots, bran, sprouted gram, and water *ad libitum* It is not our experience that rabbits do not need to be given drinking water Indeed, it is as essential to their well-being as it is to that of other laboratory animals A full-grown rabbit needs 60 to 70 c cs of water daily

A further figure may here be referred to the VALUE OF 'i', 'i' being the relative thyroid-weight on body-weight, expressed as mg per 1,000 gs of body-weight For normally-fed rabbits in this laboratory, of the range of body-weight above stated, the value of 'i' has been found to vary between

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*To be published at a later date

†It should here be emphasized that three local conditions in Coonoor—altitude, calcium-content of the soil and iodine-content of soil and vegetables grown upon it—all have an influence on the thyroid gland The height of Coonoor above sea-level is 6,000 ft , the soil is very poor in calcium, and relatively rich in iodine

100 and 141 at different seasons of the year, the *lower* figure being found in mid-summer, the *higher* in the spring, early summer and autumn Within this range of body-weight—1,500 to 2,500 gs—values of '1', in excess of 125 in summer experiments and of 176 in spring, early summer and autumn experiments, are to be regarded as definitely abnormal and indicative of thyroid enlargement These arbitrary limits are arrived at by adding one-quarter of the value of '1' to the normal value of '1' for each season

First Experiment

This experiment was carried out during the early summer May and June 1930 Its duration was 54 days

Eighteen rabbits were used They were divided into three groups of the same aggregate body-weight 8,430/40 grammes There were three males and three females in each group The animals were confined each in a separate cage under conditions of scrupulous cleanliness Distilled water for drinking purposes was provided *ad libitum* One group was fed on an exclusive diet of raw cabbage, the second on cabbage, from the same source, which had been steamed for 15 minutes in a steam-sterilizer, and the third on a control, stock diet consisting of cabbage, grass, carrots, sprouted Bengal gram and bran *

The cabbage used was locally grown on soil whose iodine-content is relatively high (average 284γ per kilogram) Fresh supplies were obtained each day, deterioration consequent on storage being thus avoided Similarly, the components of the control diet were of the freshest Cabbage was supplied *ad libitum* to Groups I and II, an account being kept of each animal's daily consumption of it (Table I)

The rabbits were killed by air-embolism on the morning of the fifty-fifth day, then thyroids were then dissected out and weighed The weights are set out in Table I

At post-mortem examination the thyroid glands of all animals fed on steamed cabbage were seen to be enlarged and congested In colour the organ had the brown, glistening appearance of a ripe, newly-shelled chestnut The isthmus was broadened, though not as a rule much thickened, and covered two or three times the usual number of tracheal rings The lobes varied in size in different animals, and in the direction of their enlargement On removal of the gland from the body it lost considerably in size consequent on drainage of blood from it Taking 176 as the value of '1,' beyond which the thyroid can be regarded as being definitely enlarged, it will be seen from Table I that all six animals in this group were goitrous

Of the six animals fed on raw cabbage four had thyroids presenting appearances similar to those just described, these were goitrous Two thyroids were not enlarged though they showed some congestion

* The average consumption of these components of the stock diet were cabbage, 220 grammes, grass, 243 grammes, carrots, 64 grammes, sprouted Bengal gram, 90 grammes, bran, 100 grammes, water 60 c cs

TABLE I

Giving details and results of the First Experiment

Group	Diet	Number of animal	Sex	Original body-weight gs	Final body-weight gs	Weight of thyroid mg	Value of 'r'	Iodine in thyroid γ per 100 mg of fresh gland γ	Iodine in urine γ per litre	Daily average consumption of cabbage gs	Co-efficient of variability of 'r'
1	2	3	4	5	6	7	8	9	10	11	12
I	Steamed cabbage	1	M	1 520	1 520	313	206			793	
		2	F	1 390	1 450	137	301			756	
		3	M	1 445	1 465	697	176		6	749	
		4	F	1 305	1 670	528	316	0·3		821	
		5	M	1,350	1 465	551	376	0·3		817	
		6	F	1 430	1 800	559	311	0·3		821	
Averages			..	1 407	1 562	514	331	0·3	6	793	26·9
II	Raw cabbage	7	M	1 385	1 395	591	126	0·3		794	
		8	F	1,315	1 580	238	151	2 1		787	
		9	M	1 540	1 535	311	221	1 8	6	775	
		10	F	1 380	1 545	323	209	0 6		783	
		11	M	1 455	1 630	233	113	1 2		776	.
		12	F	1 365	1 465	278	190	1·3		751	.
Averages				1 407	1 525	335	224	1 2	6	778	46·2
III	Control stock diet	13	M	1 430	2,110	428	203	0·5		250	..
		14	F	1,400	2 025	226	112	0 9		211	.
		15	M	1,445	1,705	207	122	3 5	9	210	..
		16	F	1 290	1,705	174	102	3 5		209	..
		17	M	1,460	1 805	284	157	0 7		214	..
		18	F	1,415	1 450	216	147	1·6		216	..
Averages				1,407	1,800	256	141	1 8	9	218	26·3

Of the six animals fed on the control diet one (No 13, Table I) showed slight enlargement and the same ripe-chestnut appearance as goitrous organs in the other two groups

One lobe of each gland was set aside for histological study and the other for determination of its iodine-content The findings in regard to the latter are set out in Table I The iodine-estimations in the first three animals were rejected since at the time they were made it was not realized that the iodine-content of the gland would be so low, and experience had not been gained as to the amount of tissue necessary for the determinations

The urine of two animals in each group was pooled, estimations of its iodine-content being made on three occasions The average of the three estimations is shown for each group in column 10, Table I The average daily consumption of cabbage by each animal is given in column 11, Table I

[The figures given for urinary iodine throughout this paper relate to 17-hour samples collected between 4 P M and 9 A M *They do not represent the total excretion of iodine in the urine* Owing to the lack of suitable metabolism cages for rabbits the method adopted for collecting the urine was as follows —The animals selected to provide the samples, were kept in their own cages from 9 A M until 4 P M during which time they were fed and watered At 4 P M they were transferred to cages specially designed for the collection of uncontaminated urine This procedure was followed for 2 or 3 days until the amount of urine (100 c c or more) needed for our purpose had accumulated In the first and second experiment two animals from each group were used to provide the samples, in the third a sample was obtained from each animal Three estimations were made of each sample, the average of the three being the figure shown in the Tables The estimations both of the iodine-content of the urines and of the thyroid glands were made by my assistant—Dr G Sankaran—by a modified A Fellenberg method (Sankaran, 1930)]

Results of the First Experiment

1 The cabbage used in this experiment had a pronounced goitrogenic action

2 Steamed cabbage was more potent and more uniform in its action than raw cabbage

3 The difference in goitrogenic activity between raw and steamed cabbage was not due to a greater consumption of the latter, but to some change brought about by steaming which either augmented the action of the goitrogenic agent or which destroyed some anti-goitrogenic substance present in cabbage (Marine *et al*, 1930)

4 The iodine-content of the thyroid gland was least in rabbits fed on steamed cabbage and greatest in the control group There was, on the average, 4 times as much iodine in the glands of rabbits fed on raw cabbage as in those of rabbits fed on steamed cabbage

5 In general there was an inverse relation between the size of the thyroid gland and its iodine-content

6 The urinary excretion of iodine was the same in the 'steamed' and in the 'raw cabbage' groups In the control group the urinary excretion of iodine was higher than in either of the other two

7 The histological features of the enlarged glands in this experiment were (*a*) hyperplasia, (*b*) absence or paucity of colloid, and (*c*) engorgement These appearances are illustrated in Plate LXXX, figs 19 and 20

Second Experiment.

This experiment was carried out, during the months of July and August 1930, under the same conditions as those of the first one Ninety rabbits were used They were divided into 15 groups of 6 and fed as follows —

Group	IV	on raw cabbage
„	V	on raw cabbage and iodine water
„	VI	on steamed cabbage
„	VII	on steamed cabbage and iodine water
„	VIII	on steamed cabbage and thyroxine,
„	IX	on steamed cabbage soaked in chlorine water
„	X	on a control stock diet consisting of raw cabbage, grass, carrots, sprouted gram and bran
„	XI	on steamed cabbage soaked in brine
„	XII	on steamed cabbage and radiostoleum (B D H)
„	XIII	on steamed cabbage and carrots
„	XIV	on steamed cabbage and sprouted gram
„	XV	on steamed cabbage and bran
„	XVI	on steamed cabbage and grass
„	XVII	on a control diet consisting of steamed cabbage, grass, carrots, sprouted gram and bran
„	XVIII	on raw cabbage and carrots

All groups were provided with distilled, drinking water *ad libitum* Iodine–1 5 mg per litre–was added to the drinking water of Groups V and VII, they were housed well away from the other groups Group VIII was given 2 tablets of thyroxine, each containing 0 8 mg, amongst the six animals twice a week, the drug was mixed with mashed cabbage and given first thing in the morning Radiostoleum (B D H)–one drop per rabbit per day–was administered in the same way to the animals in Group XII The 'chlorine water,' used in Group IX, was freshly prepared, the steamed cabbage being soaked in it overnight Similarly, the steamed cabbage was soaked overnight in a concentrated solution of sodium chloride before being given to the animals in Group XI The carrots used in the diets of Groups X, XIII, XVII and XVIII were given raw, 100 grammes being presented to each animal daily, of this ration the average consumption was about 70 grammes The other food-materials used in the experiment–grass, sprouted gram and bran–were provided *ad libitum*, the daily average amounts eaten being of grass, approximately 250 grammes, of sprouted gram, 90 grammes, and, of bran, 100 grammes In all groups the cabbage, whether raw or steamed, was provided *ad libitum*

The results are set out in Table II, together with the daily average cabbage consumption, the iodine-content of the thyroid glands and the urinary excretion of iodine by each group The data in Table II are expressed as averages in Table III

TABLE II

Giving details and results of the Second Experiment

Group	Diet	Number of animal	Sex	Original body-weight gs	Final body-weight gs	Weight of thyroid mg	Value of 'r'	Iodine in thyroid γ per 100 mg of fresh gland γ	Iodine in urine γ per litre	Daily average consumption of cabbage gs
1	2	3	4	5	6	7	8	9	10	11
IV	Raw cabbage	19	M	1,650	1,665	419 0	252	1 1		792
		20	M	1 675	1 815	228·2	126	4 7		775
		21	M	1,515	1,425	243·8	171	2 4		785
		22	F	1,690	1,790	326·4	182	1 6		776
		23	F	2 020	1,855	240·6	130	1 4		772
		24	F	1,750	1 725	139·2	81	7·6		784
Averages				1 717	1,713	290	157	3·03	9·3	781
V	Raw cabbage and iodine	25	M	1,700	1,825	154 8	85	6 0		747
		26	M	1,650	1,740	196 6	113	1·2		764
		27	M	1,630	1,565	150·4	96	6·3		772
		28	F	1,720	1,730	156 4	90	2 9		753
		29	F	1,770	1,860	169·2	91	3·1		765
		30	F	1,830	1 980	167 2	84	4 4		758
Averages				1,717	1,783	217	93	3·98	28·0	760

TABLE II—*contd*

Giving details and results of the Second Experiment

Group	Diet	Number of animal	Sex	Original body-weight gs	Final body-weight gs	Weight of thyroid mg	Value of 'r'	Iodine in thyroid γ per 100 mg of fresh gland γ	Iodine in urine γ per litre γ	Daily average consumption of cabbage gs
1	2	3	4	5	6	7	8	9	10	11
VI	Steamed cabbage	31	M	1 380	1 465	316·1	216	7 8		800
		32	M	1,650	1 670	307 0	181	1 7		814
		33	M	1 905	2 005	116 6	73	14 0		815
		34	F	1,640	1 830	150 8	82	2 6		808
		35	F	1 910	1,850	119 6	81	8·5		794
		36	F	1 815	2,020	266·1	132	2·6		796
Averages				1,717	1,807	255	128	6 20	12·7	805
VII	Steamed cabbage and iodine	37	F	1,530	1,905	212·2	127	2 5		795
		38	M	1,620	1,610	170 6	106	4 7		773
		39	M	1,780	1,780	200 6	113	2 2		815
		40	M	1,720	1,900	168·6	89	14 4		789
		41	F	1,760	2,000	160·4	80	9·2		815
		42	F	1,890	2,165	128 2	59	5 5		814
Averages				1,717	1,893	178	96	6·42	23·3	800
VIII	Steamed cabbage and thyroxine	43	F	1,900	2,015	103·6	51	24 0		806
		44	F	1,730	1,830	95·4	52	19 8		798
		45	M	1,750	1,815	177 6	98	18 8		815
		46 *	F	1,750	1,475	79·4	54	?		772
		47	M	1,740	1,970	146 4	74	21 0		808
		48	F	1,430	2,165	172·8	79	47 0		814
Averages				1,717	1,878	129·2	68	26 1	110·0	802

* Died on the 49th day

TABLE II—*contd*
Giving details and results of the Second Experiment

Group	Diet	Number of animal	Sex	Original body-weight gs	Final body-weight gs	Weight of thyroid mg	Value of 'r'	Iodine in thyroid γ per 100 mg of fresh gland	Iodine in urine γ per litre	Daily average consumption of cabbage gs
1	2	3	4	5	6	7	8	9	10	11
IX	Steamed cabbage and chlorine water	49	M	1,800	1,840	166·8	91	2·6		817
		50	M	1,820	1,850	373·2	202	0·8		816
		51	M	1,810	1,815	221·2	122	0·7		810
		52	F	1,600	1,755	188·0	107	1·7		814
		53	F	1,800	1,835	185·4	101	1·1		819
		54	F	1,470	1,480	226·8	153	0·8		814
Averages				1,717	1,763	226·9	129	1·28	25·0	815
X	Control diet raw cabbage	55	F	1,350	1,660	160·8	97	2·4		319
		56	F	1,780	1·900	183·4	96	4·1		313
		57	F	1,860	1,845	164·8	89	5·7		311
		58	M	1,840	1,975	214·0	108	7·5		311
		59	M	1,370	1,665	190·2	114	1·7		319
		60	M	2,100	2,440	230·0	94	3·7		325
Averages				1,717	1,914	190·5	100	4·18	15·3	316
XI	Steamed cabbage and sodium chloride	61	F	1,850	1,940	201·0	104	4·9		827
		62	F	1,840	1,840	235·4	128	2·0		816
		63	F	1,640	1,415	256·2	181	1·5		812
		64	M	1,370	1,570	260·0	166	2·0		807
		65	M	2,000	2,330	237·8	102	2·2		808
		66	M	1,600	1,655	542·6	328	0·6		819
Averages				1,717	1,792	288·8	168	1·87	12·0	815

TABLE II—*contd*
Giving details and results of the Second Experiment

Group	Diet	Number of animal	Sex	Original body-weight gs	Final body-weight gs	Weight of thyroid mg	Value of 'r'	Iodine in thyroid γ per 100 mg of fresh gland	Iodine in urine γ per litre	Daily average consumption of cabbage gs
1	2	3	4	5	6	7	8	9	10	11
XII	Steamed cabbage and radiostoleum	67	M	2,000	1,975	321·6	163	1 5		826
		68	F	2,290	2,200	319·8	145	1 1		825
		69	M	1,595	1,815	322 0	177	0·8		845
		70	F	1,255	1,610	217 6	133	1 5		842
		71	M	1 635	1 820	290·2	159	2·8		838
		72	M	1,525	1,535	330·8	215	1 1		845
Averages				1,717	1,831	300·0	165	1·47	9·3	837
XIII	Steamed cabbage and carrots	73	M	1,520	1,630	114 4	70	4 6		750
		74	F	1,440	1,775	232·6	131	2·2		758
		75	M	1,620	1,740	245 4	141	1 7		760
		76	F	1,380	1,840	287 0	156	0·8		759
		77	M	1,550	1,595	169·6	106	2·8		766
		78	F	1,600	1 540	128 6	83	3·5		768
Averages				1,518	1,687	196·3	115	2·60	21 0	760
XIV	Steamed cabbage and sprouted gram	79	M	1,580	2,135	251 8	118	1 5		758
		80	F	1,355	1,690	209 4	124	1 0		757
		81	M	1,870	2,250	253·6	113	1 1		775
		82	F	1,445	1,890	305 6	162	0·5		762
		83	M	1,430	1,695	222·0	131	1 0		763
		84	F	1,430	1,730	182·6	106	1 7		762
Averages				1,518	1,898	237 5	126	1 13	11 3	763

TABLE II—*contd*
Giving details and results of the Second Experiment

Group	Diet	Number of animal	Sex	Original body weight gs	Final body-weight gs	Weight of thyroid mg	Value of 'r'	Iodine in thyroid γ per 100 mg of fresh gland γ	Iodine in urine γ per litre	Daily average consumption of cabbage gs
1	2	3	4	5	6	7	8	9	10	11
XV	Steamed cabbage and bran	85	M	1,500	1,775	305·8	172	0·7		757
		86	F	1,755	2,105	263·2	125	0·8		718
		87	M	1,405	1,600	464·0	290	1·0		767
		88	F	1,480	1,720	347·2	202	1·6		774
		89	M	1 500	1,900	211·6	111	3·0		765
		90	F	1,470	1 400	306·0	218	0·7		759
Averages				1,518	1,750	316·3	186	1·3	12·0	757
XVI	Steamed cabbage and grass	91	M	1,530	1,355	287·8	212	0·8		762
		92	F	1,115	1,470	190·6	129	4·2		760
		93	M	1,370	1,680	129·6	77	4·8		760
		94	F	2,325	2,305	279·2	121	2·2		758
		95	M	1,595	1,800	216·4	120	2·4		773
		96	F	1,375	1,765	168·4	95	5·0		737
Averages				1,552	1,729	212·0	126	3·23	21·7	758
XVII	Control diet Steamed cabbage	97	M	1,495	2,000	203·8	102	3·6		430
		98	F	1,885	2,420	129·2	53	9·6		426
		99	M	1,735	1,910	135·8	71	7·7		431
		100	F	1,395	1,580	156·2	99	1·9		425
		101	M	1,350	1,540	417·2	271	0·8		440
		102	F	1,280	2,115	223·4	106	2·0		444
Averages				1,518	1,928	210·9	117	4·27	12·0	433

TABLE II—*concld*

Giving details and results of the Second Experiment

Group	Diet	Number of animal	Sex	Original body-weight gs	Final body-weight gs	Weight of thyroid mg	Value of 'r'	Iodine in thyroid γ per 100 mg of fresh gland	Iodine in urine γ per litre	Daily average consumption of cabbage gs
1	2	3	4	5	6	7	8	9	10	11
XVIII	Raw cabbage and carrots	103	M.	1 710	1 630	2790	171	1 0		671
		104	F	1 470	1 590	2100	132	1 6		656
		105	M	1 685	1 725	1882	109	2 3		687
		106	F	1 130	1 100	1832	131	3 6		690
		107	M	1 265	1 165	317 8	237	1 0		683
		108	F	1 580	1,815	1110	79	8·6		721
Averages				1,528	1,601	225 1	143	3 02	10 7	685

TABLE III

Giving results of the Second Experiment as averages

Diets arranged in ascending order of value of 'r'

Group	Diet	Increase or decrease in body-weight gs	Mean value of 'r'	Number of goitres	Mean glandular iodine γ per 100 mg γ	Mean urinary iodine γ per litre γ
1	2	3	4	5	6	7
VIII	Steamed cabbage + thyroxine	+ 161	68	0	26 10	110·0
V	Raw cabbage + iodine	+ 66	93	0	3 98	28·0
VII	Steamed cabbage + iodine	+ 176	96	0	6 42	23·3
X	Control diet raw cabbage	+ 197	100	0	4 18	15 3
XIII	Steamed cabbage + carrots	+ 169	115	2 (v s)	2 60	21 0
XVII	Control diet steamed cabbage	+ 410	117	1	4 27	12 0
XIV	Steamed cabbage + sprouted gram	+ 480	126	1 (v s)	1 13	11 3
XVI	Steamed cabbage + grass	+ 177	126	1	3 23	21 7
VI	Steamed cabbage only	+ 90	128	2	6 20	12·7
IX	Steamed cabbage in Cl water	+ 46	129	2 (1 v s)	1 28	25·0
XVIII	Raw cabbage + carrots	+ 76	143	2	3·02	10·7
IV	Raw cabbage only	— 4	157	3	3 03	9·3
XII	Steamed cabbage + radiostoleum	+ 114	165	5 (3 v s)	1 47	9·3
XI	Steamed cabbage + NaCl	+ 75	168	3 (1 v s)	1 87	12 0
XV	Steamed cabbage + bran	+ 232	186	4	1 30	12·0

v s = Very small

The statistical constants relating to this experiment are set out in Table IV

TABLE IV

Showing the statistical constants in the Second Experiment

Group or groups	Animal numbers	Diet	Mean body-weight gs	Mean thyroid-weight mg	Mean value of 'r'	Co-efficient of variability of 'r'
IV	19–24	Raw cabbage	1 713	290 0	157 0	37·5
V	25–30	Raw cabbage + iodine	1,783	217 0	93·0	13 2
VI	31–36	Steamed cabbage	1 807	255·0	128 0	47 2
VII	37–42	Steamed cabbage + iodine	1,893	178 0	96 0	24 0
VIII	43–48	Steamed cabbage + thyroxine	1 878	129·2	68·0	28·0
IX	49–54	Steamed cabbage + chlorine H_2O	1,763	226 9	129·0	32·2
X	55–60	Control raw cabbage	1,914	190·5	100·0	9 3
XI	61–66	Steamed cabbage + sodium chloride	1,792	288·8	168·0	50·3
XII	67–72	Steamed cabbage + radiostoleum	1,831	300·0	165 0	17 5
XIII	73–78	Steamed cabbage + carrots	1,687	196·3	115 0	38 4
XIV	79–84	Steamed cabbage + sprouted gram	1,898	237 5	126 0	15 7
XV	85–90	Steamed cabbage + bran	1,750	316·3	186 0	42·7
XVI	91–96	Steamed cabbage + grass	1,729	212 0	126·0	37 0
XVII	97–102	Control Steamed cabbage	1 928	210·9	117 0	66 9
XVIII	103–108	Raw cabbage + carrots	1 604	225 4	143 0	38·4

Notes—(1) The degree of goitrogenic action is indicated by high value of 'r', the degree of anti-goitrogenic action by low value of 'r'

(2) The uniformity of goitrogenic or anti-goitrogenic action is indicated by low co-efficient of variability of 'r'

Results of the Second Experiment

(1) In this experiment the normal size of the thyroid gland, as indicated by the mean value of 'r' in the two groups (X and XVII) fed on well-balanced, control diets, was 108 mg per kilogram of body-weight Glands exceeding this size by more than one-quarter (i e, in excess of 135 mg) are considered to have been enlarged On this basis–admittedly an arbitrary one–the number of

goitres in each group is shown in column 5, Table III Twenty-six out of ninety animals were goitrous, or 28 8 per cent Four groups were free from goitre those receiving thyroxine, iodine and the control diet containing raw cabbage The incidence of goitre in the remaining eleven groups was 39 4 per cent Of the 26 goitres 8 were very small, these are indicated in column 5, Table III, by the letters 'v s,' the remaining 18 were of moderate size, two or three times that of the normal organ Photographs showing the glands in the more important groups are reproduced in Plates LXXII to LXXVII, figs 1 to 12

An unexpected result of this experiment was that the thyroid of males was, in general, larger than that of females, the mean 'r'-value in the former being 152 and in the latter 124 as calculated on the original body-weight of the animals, and 140 and 111 respectively, as calculated on their final body-weight Associated with this difference in size of the gland in the two sexes there is a significant difference in the iodine-content of the gland, this being in inverse relation to the size of the organ 1 58 γ per 100 mg of fresh gland in males, and 2 46 γ in females There were 19 goitres in males and 7 in females, an unusual sex incidence

(2) In all groups except one (IV, 'Raw cabbage only') the body-weight increased during the 54 days of the experiment, the average increase ranging from 46 to 480 grammes In contrasting the size of the thyroid gland in the different groups the effects of the various diets on the body-weight have to be taken into consideration The values of 'r' were accordingly calculated against the *original body-weights* of the animals and contrasted with those arrived at when the *final body-weights* are taken as the basis of the calculations On the former basis the various groups, arranged in ascending order of values of 'r,' assume a somewhat different order to that shown in Table III, thus —

Group	Diet	Mean value of 'r' calculated on	
		Original body-weight	Final body-weight
VIII	Steamed cabbage + thyroxine	75	68
V	Raw cabbage + iodine	97	93
VII	Steamed cabbage + iodine	104	96
X	Control diet raw cabbage	111	100
VI	Steamed cabbage only	124	128
XIII	Steamed cabbage + carrots	129	115
IX	Steamed cabbage soaked in chlorine water	132	129
XVI	Steamed cabbage + grass	137	126
XVII	Control diet steamed cabbage	139	117
XVIII	Raw cabbage + carrots	147	143
IV	Raw cabbage only	155	157
XIV	Steamed cabbage + sprouted grain	156	126
XI	Steamed cabbage + sodium chloride	169	168
XII	Steamed cabbage + radiostoleum	175	165
XV	Steamed cabbage + bran	208	186

By whichever criterion the values of 'r' are judged there were certain substances whose action was antagonistic to thyroid enlargement, and certain others whose action was definitely favourable to it In the former category are thyroxine and iodine, in the latter, sodium chloride, radiostoleum and bran

(3) The cabbage used in this experiment was less potent to cause goitre than that used in the first experiment The mean value of 'r' in the first experiment was 224 for animals fed on raw cabbage, whereas in the present one it was 157 Associated with the larger size of the gland in the first experiment there was a much lower content of iodine in the thyroid gland (1 2 as compared with 3 03 γ), and a lower excretion of iodine in the urine (6 as compared with 9 3 γ per litre) These figures suggest that the cabbage used in the second experiment was richer in iodine than that used in the first The difference in the goitrogenic potency may, however, have been due to a greater concentration of goitrogenic substance in the early summer than in the late summer cabbage, or to a lesser content of anti-goitrogenic substance other than iodine (Marine *et al*, 1930) in the one than in the other Owing to the larger amount of cabbage used daily in the second experiment we had to go further afield for it, it was obtained from places within a 10-mile radius of Coonoor The place of origin and growth of the cabbage may not be without influence in determining its goitrogenic potency Apart from these considerations the question of season *per se*, in favouring or disfavouring the genesis of goitre, has to be borne in mind

(4) In this experiment steaming did not increase the goitrogenic potency of the cabbage, on the contrary, it reduced this potency (Table III)–a result directly opposed to that yielded by the first experiment There were certain differences in the method of steaming in the two experiments in the first, the amount of cabbage to be steamed was small and the steaming was done in a small steam-sterilizer, in the second, the amount of cabbage to be steamed was large and the steaming was done in a large autoclave, the lid of which was not screwed down during the 15 minutes' steaming It may be that this difference in method of steaming accounts for the different effect of the cabbage in the two experiments However this may be, the effect of steaming was as definitely favourable to goitre-production in the one experiment as it was antagonistic to it in the other

(5) If the values of 'r,' as determined by calculation from the *final body-weights* of the animals, be taken as the criteria for comparison, then carrots, grass and sprouted Bengal gram had each a slight anti-goitrogenic action, but if the values of 'r,' as determined by calculation from the *original body-weights*, be used as the criteria for comparison then carrots alone exercised any anti-goitrogenic action and then only when the basis of the diet was raw cabbage One effect of carrots, which appears to be fairly definite, is that when they were added to the diet of steamed cabbage they caused a loss of iodine from the gland and a considerably increased excretion of iodine in the urine and, at the same time, a slight reduction in size of the thyroid gland (compare Groups XIII

and VI, Table III) These effects on iodine-metabolism were not observed when carrots were added to the diet of raw cabbage though here also a slight reduction in size of the thyroid occurred (compare Groups IV and XVIII, Table III) Grass had much the same effects as carrots when added to the diet of steamed cabbage Sprouted gram, when added to the diet of steamed cabbage, caused a marked drop in the iodine-content of the gland without materially altering the rate of iodine-excretion in the urine

(6) Bran was definitely favourable to goitre-production When added to the diet of steamed cabbage it caused a marked fall in the iodine-content of the gland without any significant change in the urinary excretion of iodine

(7) Of the chemical substances used in this experiment two–thyroxine and iodine–had a very marked anti-goitrogenic action, two–sodium chloride and radiostoleum–had an action favourable to goitre-production, and, one–chlorine–a neutral action in so far as the size of the thyroid gland was concerned

Thyroxine, when added to the diet of steamed cabbage, caused the thyroid gland to be of small size, pale in colour, and of high iodine-content, while the urinary excretion of iodine by the animals receiving it was high (110 γ per litre) On section the glands were usually full of colloid, but there were exceptions to this rule in which the amount of colloid was relatively scanty (Plate LXXXIII, figs 31, 32) In general the higher the iodine-content of the gland the more abundant was the colloid store (*vide* Table II)

Iodine, when added to the drinking water of animals fed either on raw or on steamed cabbage, did not greatly increase the iodine-content of the gland though it markedly increased the urinary excretion of iodine (Tables II and III) The amount of colloid seen in the glands of these animals, on microscopical examination, was variable and by no means large (Plate LXXXIII, figs 29, 30)

Chlorine had a very remarkable effect the thyroids of rabbits fed on steamed cabbage which had been soaked in fresh chlorine water overnight did not differ in size from those of rabbits fed on steamed cabbage which had not been subjected to this treatment (compare Groups VI and IX, Table III), but they contained much less iodine while the urinary excretion of iodine was doubled Chlorine appeared, therefore, to cause a loss of iodine from the body without increasing the size of the thyroid gland The histological appearances of the glands in this group (IX) are shown in Plate LXXXII, fig 26

Sodium chloride enhanced the goitre-producing action of steamed cabbage, it diminished the amount of iodine in the gland without altering the amount excreted in the urine (Plate LXXXII, fig 25)

Radiostoleum also enhanced the goitre-producing action of steamed cabbage, it reduced the iodine-content of the gland as well as slightly reducing the amount of iodine excreted in the urine This action of fat-soluble vitamins in favouring the development of goitre is in sharp contrast to previous experience in this laboratory (McCarrison, 1930) which has indicated that deficiency

of these vitamins is favourable to the genesis of *lymph-adenoid* goitre The response of the gland to goitrogenic agents is, however, different in the two cases For whereas in the one (as in the present experiment) it is that of a physiologically perfect organ resulting in multiplication of efficient glandular elements in the other (as when fat-soluble vitamins are lacking in the diet) it is that of a physiologically inefficient organ resulting in lymphocytic infiltration and atrophy of the parenchyma cells In the one the 'goitre' is of a hypertrophic type (Plate LXXXII, fig 27) and likely only to undergo degenerative change as a result of overstrain, in the other the 'goitre' is degenerative from the outset It would appear therefore, that while an abundance of fat-soluble vitamins in the diet may, in circumstances such as those of the present experiment, actually favour the development of 'hypertrophic goitre' it will disfavour the development of 'lymph-adenoid goitre' since the functional efficiency of the glandular epithelium is largely dependent on a sufficient provision of these vitamins in the food (McCarrison, 1930)

Third Experiment

This experiment was carried out during the months of September and October 1930, its conditions and duration were the same as those in the first and second experiments Twenty-four rabbits were used, they were divided into four groups and fed as follows —

Group XIX on raw cabbage only
„ XX on raw cabbage soaked in chlorine water
„ XXI on raw cabbage plus manganese chloride
„ XXII on raw cabbage plus thymol

All were given distilled, drinking water *ad libitum*

The chlorine water used in Group XX was freshly prepared, the raw cabbage being immersed in it overnight

The manganese chloride was administered to the animals in Group XXI in the following way a solution of $MnCl_2$, in the proportion of 2 grammes to the litre of distilled water, was made, of this 1 c c (equal to 2 mg of $MnCl_2$) was smeared on a cabbage leaf and presented to each animal first thing in the morning when it was hungry Little difficulty was experienced in getting them to eat the medicated leaves

The thymol was administered to the animals in Group XXII in the following way The daily dose of the drug was 6 grains, this amount was weighed out for each animal, and ground up into a paste with a drop or two of water It was then smeared on a cabbage leaf, the medicated leaves being presented to the animals first thing in the morning During the first few days of the experiment some difficulty was experienced in getting the animals to eat the thymol-smeared leaves, but by keeping them hungry until they did eat them this difficulty was gradually overcome By this method of administration some of the thymol was apt to be lost as the paste dried, and in consequence

certain animals ingested less thymol than others But in general the method was successful

The results of this experiment are set out in Table V Photographs illustrating them are shown in Plates LXXVIII and LXXIX, figs 13 to 16

TABLE V

Giving details and results of the Third Experiment

Group	Diet	Number of animal	Sex	Original body-weight gs	Final body-weight gs	Thyroid weight mg	Value of 'r'	Iodine in thyroid γ per 100 mg of fresh gland γ	Iodine in urine γ per litre γ	Daily average consumption of cabbage gs
1	2	3	4	5	6	7	8	9	10	11
XIX	Raw cabbage	109	F	1,555	1,610	365 6	227	0·9	1 0	646
		110	M	1,620	1,650	343·6	208	0·6	0·9	637
		111	M	1 570	1,210	431 C	357	0·1	0 4	663
		112 *	F	1 505	810	355 2	423	0 4	0·5	652
		113	F	2 275	2,080	281·8	137	1·5	0·4	657
		114	F	1,475	1,790	497 6	278	0·7	0·8	637
Averages				1,667	1,530	379·7	272·0	0·7	0·67	648
XX	Raw cabbage in chlorine water	115 †	M	1,700	1 400	238·8	171	1·7	0 9	582
		116	M	1,760	1,900	399·2	210	0·2	0·8	643
		117	M	1,720	1,830	838·8	458	0·13	0 7	649
		118	F	1,470	1 600	465 2	291	0 2	0·3	634
		119	F	1,700	1,780	356·4	200	0·13	0·4	661
		120	F	1 650	1,380	414·0	300	0·3	0·4	648
Averages				1,667	1,648	452·1	271 7	0·44	0·58	636
XXI	Raw cabbage in manganese chloride	121 ‡	M	1,495	1,400	254 2	181	0·4	0·5	623
		122	M	1 780	1,840	220·4	120	0 9	0·6	647
		123	M	2,140	2,080	150·0	72	5·1	0 3	662
		124	F	1 470	1,700	263·2	155	1 2	0·4	659
		125	F	1,575	1,650	242 6	147	0·7	0·4	649
		126	F	1,540	1 675	172 8	103	1 8	0·3	644
Averages				1 667	1,724	217·2	129·7	1 7	0·42	647
XXII	Raw cabbage and thymol	127	M	1,800	1,650	135 4	82	3·4	0 5	640
		128	M	1,865	1,660	406 6	245	0 6	0·9	657
		129	F	1,610	1,885	317 0	168	0·4	0·6	648
		130	F	1,560	1,740	132 4	76	9 0	1·4	653
		131	F	1 595	1,655	92·6	56	11 0	2·3	656
		132	F	1,570	1,600	259 8	162	0·7	0·5	666
Averages				1,667	1,698	224 0	131·5	4·2	1 03	653

* Died on 50th day of experiment

† Killed on 43rd day of experiment

‡ Killed on 47th day of experiment

The statistical constants relating to this experiment are set out in Table VI, and the significance of their differences in Table VII

TABLE VI

Showing statistical constants in the Third Experiment

Group	Animal numbers	Diet	Mean body-weight gs	Mean thyroid-weight mg	Mean value of 'r' with standard error of same	Co-efficient of variability 'r'
XIX	109–114	Raw cabbage only	1 530	379·7	272·0 ± 42 6	38 4
XX	115–120	Raw cabbage + chlorine water	1 648	452·1	271 7 ± 42 5	38·6
XXI	121–126	Raw cabbage + manganese chloride	1,724	217·2	129·7 ± 16 2	30·5
XXII	127–132	Raw cabbage + thymol	1 698	224 0	131 5 ± 28·5	51 4

TABLE VII

Showing the significance of the differences

Universes contrasted	Actual difference of 'r' values	Standard error of difference	P	Significance
XIX *versus* XXI	142·3	45 6	1 2	Significant
XIX *versus* XXII	140 5	51 2	2·6	Significant

Results of the Third Experiment

(1) The animals in this experiment ate less cabbage than in either of the other two, but the average daily consumption by each of the four groups was approximately the same The differences in the average size of the thyroid gland ('r' column 8, Table V) were not due, therefore, to differences in the amounts of cabbage consumed

(2) The goitre-producing potency of the cabbage used in this experiment was considerably greater than that of the cabbage used in either of the other experiments, the mean value of 'r' being 279, as compared with 224 and 157 in the first and second experiment respectively

(3) The immersion of the raw cabbage in fresh chlorine water did not alter its goitrogenic activity, the mean values of 'r' in Groups XIX and XX being approximately the same 279 and 271 respectively But the average iodine-content of the gland and the average urinary excretion of iodine were somewhat less than in the rabbits fed on the untreated cabbage

(4) Taking the mean value of 'i' in well-fed stock rabbits, of the same range of body-weight as those in the present experiment, as being 141 during the autumn months in Coonoor, then it may safely be assumed that values of 'i' exceeding this figure by more than one-quarter (i.e., exceeding 176) are indicative of goitrous glands. On this basis 5 of the glands in Group XIX had values of 'i' in excess of 176, while 5 in Group XX, 1 in Group XXI, and 1 in Group XXII had values of 'i' in excess of this figure. There were thus 12 cases of goitre amongst the 24 animals, or 50 per cent. Ten of the goitres occurred in Groups XIX and XX while only two occurred in the groups receiving manganese chloride or thymol. At post-mortem examination these goitres were readily recognized by their size and by their glistening, ripe-chestnut appearance. On removal from the body they lost considerably in size owing to drainage of blood from them. Their macroscopical and microscopical appearances are shown in Plates LXXVIII and LXXIX, figs. 13 to 16, Plate LXXXII fig. 28, and Plate LXXXIV figs. 33 to 36.

(5) *Manganese chloride* exercised a significant anti-goitrogenic action, the mean value of 'i' in Group XXI being less than half of that in Group XIX. In only one case (No. 121) was the value of 'i' in excess of 176 and then only to a very slight extent. The administration of manganese chloride prevented, to a considerable extent, the loss of iodine from the gland which is induced by the cabbage diet, there being, on the average, nearly $2\frac{1}{2}$ times as much iodine in the glands of animals receiving manganese chloride as in those of animals not receiving it. Associated with this higher content of glandular iodine there was a somewhat lower excretion of iodine in the urine.

(6) *Thymol* also exercised a significant anti-goitrogenic action, though it was less uniform than that of manganese chloride. The co-efficient of variability in the thyroid size was 51·4 in the thymol group as compared with 30·5 in the manganese group. There was only one gland (No. 128) in this group (XXII) whose 'i'-value was in excess of 176, this gland was goitrous, the other 5 were not. It will be observed that the iodine-content of the thyroid in this group varied within wide limits (0·4 to 11·0 γ), the smaller glands having a high and the larger glands a low iodine-content. The mean iodine-content of the thyroids was six times greater in rabbits receiving thymol than in those not receiving it, the urinary excretion of iodine was also considerably higher.

Anti-Goitrogenic Action of Iodine and Thyroxine

It will be observed from Table IV and from Plates LXXIII and LXXIV, figs. 4 and 6, that the anti-goitrogenic action of iodine was a uniform one. The co-efficient of variability in size of the thyroid glands of animals receiving iodine (Groups V and VII) was low averaging 18·6, as compared with 37·5 in the group fed on raw cabbage only and with 47·2 in the group fed on steamed cabbage only (second experiment). This low co-efficient of variability is paralleled in two other groups in this series the 'radiostoleum' group (XII)

and the 'sprouted gram' group (XIV) in which the respective co-efficients were lower still, being 17 5 and 15 7 respectively In these three groups the action of the substances added to the cabbage diet–iodine, radiostoleum and sprouted gram–was of like uniformity, but whereas iodine was antagonistic to goitre-formation, radiostoleum was favourable to it and sprouted gram was neutral

The anti-goitrogenic action of thyroxine was less uniform that that of iodine, the co-efficient of variability in size of the glands of animals receiving thyroxine being 28 0, as compared with 18 6 in animals receiving iodine Similarly, the histological features of the glands were more variable, some showing an abundance of colloid (Plate LXXXIII, fig 31), others relatively little (Plate LXXXIII, fig 32) although in all (Table II) the iodine-content of the gland was relatively high, suggesting that iodine in considerable quantity was present in the glandular cells This variableness is no doubt to be attributed to difierences in the absorption of thyroxine by different individuals, whereas iodine in the form of iodized water is, presumably, equally well absorbed by all

An interesting post-mortem finding in one of the thyroxine-fed animals must be recorded This animal (No 46) died on the forty-ninth day of the experiment A severe enteritis was present, the left auricle contained a large fibrinous clot resistant to cutting by the scissors, the cardiac musculature was much engorged, as were its superficial vessels The bladder was greatly distended with urine which was found to contain no less than 2,500 γ of iodine per litre, or 23 times as much as that in the urine voided by animals which survived the experiment

Anti-Goitrogenic Action of Manganese Chloride

It is evident from the results of the third experiment that manganese chloride exercises a profound effect on the thyroid gland In previous papers (McCarrison, 1928, 1929) attention was drawn to its action in preventing *lymph-adenoid goitre,* the results of the present experiment indicate that it is also capable of preventing the goitres induced in rabbits by a cabbage diet These observations lend support to the principles upon which Dr Herbert Nott (1925) has based his 'thyroid-manganese treatment', they suggest that manganese chloride may have a place in the treatment of thyroid disorders

From a chemical point of view the similarity in the anti-goitrogenic action of iodine and manganese chloride is not surprising Manganese and the halogens are in the same group in the Periodic Table, a certain similarity in chemical behaviour is, therefore, likely to be exhibited by iodine and manganese

The post-mortem appearances of the thyroid glands of rabbits receiving manganese chloride were in striking contrast to those seen in rabbits not receiving it For whereas in the latter the gland was enlarged, engorged, and of a glistening ripe-chestnut colour, in the former it was small, pale and almost indistinguishable in colour from the pale muscles overlying it In this

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Explanation of Plate LXXII

Fig 1 Thyroid glands of rabbits (55 to 60) fed on the control, stock diet consisting of the raw cabbage used in the second experiment together with grass, sprouted gram, carrots and bran None are goitrous

„ 2 Thyroid glands of rabbits (91 to 96) fed on steamed cabbage and grass One is goitrous

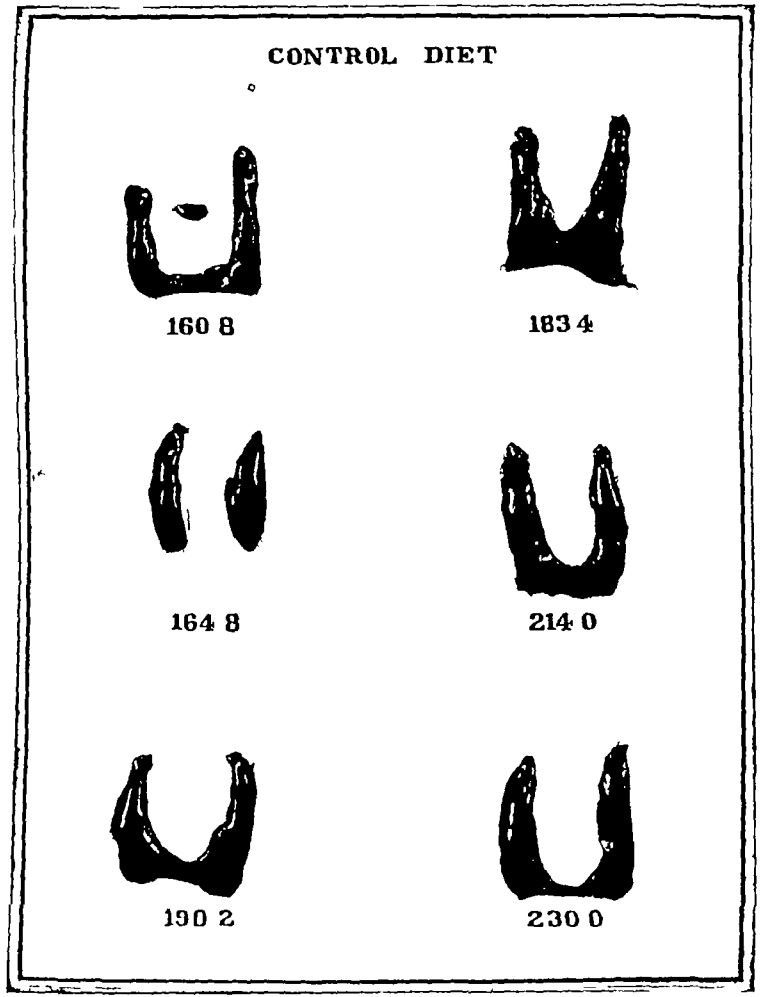

CONTROL DIET
160 8
183 4
164 8
214 0
190 2
230 0

Fig 1

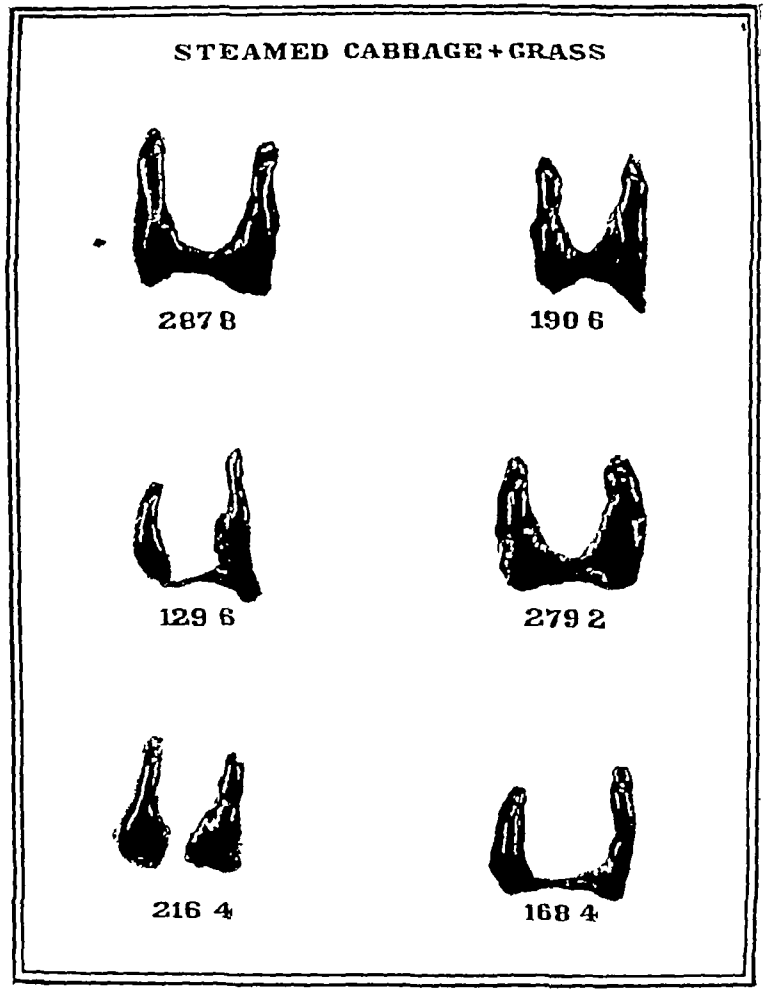

STEAMED CABBAGE+GRASS
287 8
190 6
129 6
279 2
216 4
168 4

Fig 2

Explanation of Plate LXXIII

Fig 3 Thyroid glands of rabbits (19 to 24) fed on raw cabbage only Three are goitrous

„ 4 Thyroid glands of rabbits (25 to 30) fed on raw cabbage and iodine water None are goitrous Compare with Fig 3

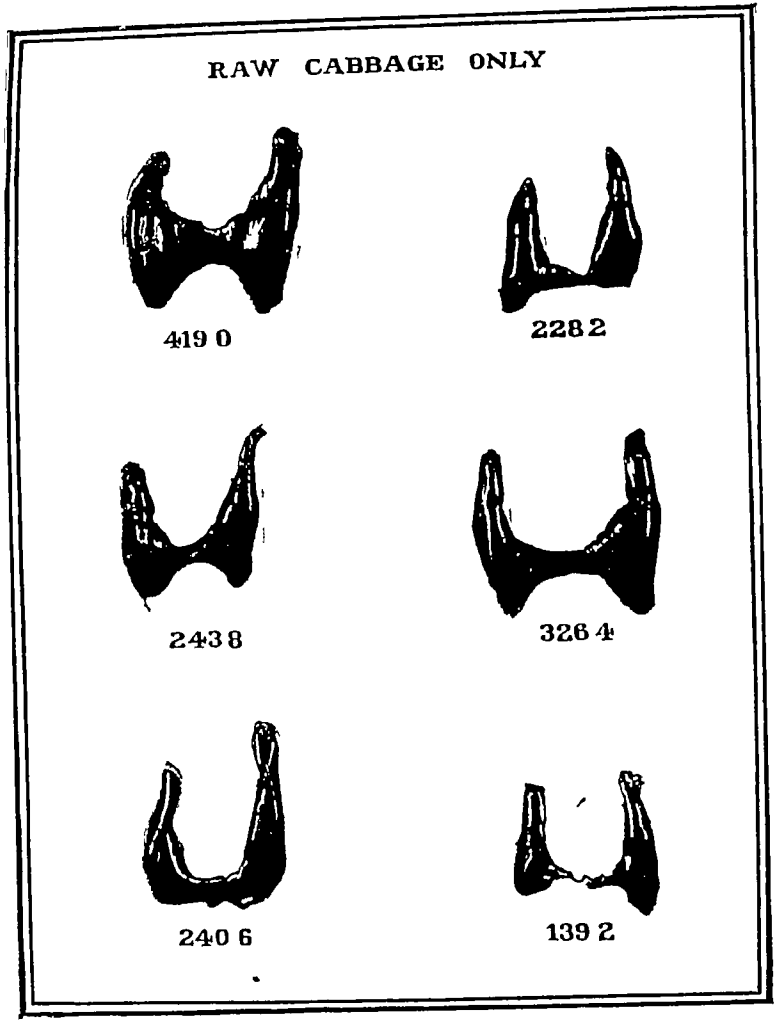

RAW CABBAGE ONLY
419 0
228 2
243 8
326 4
240 6
139 2

Fig 3

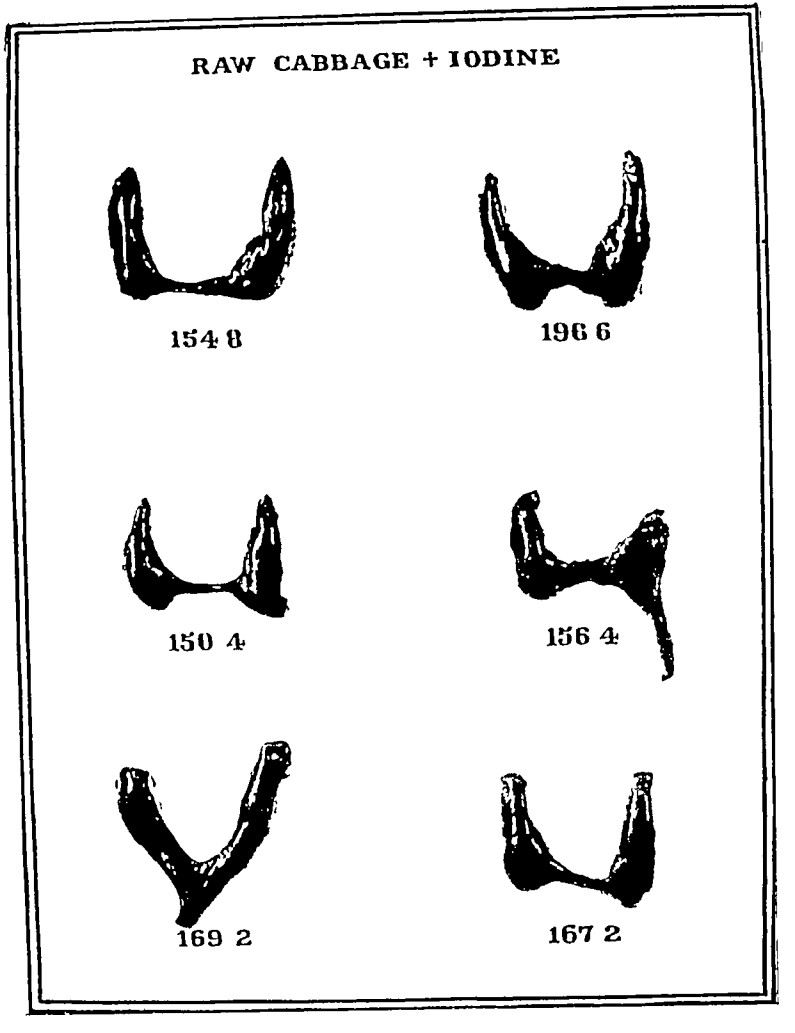

RAW CABBAGE + IODINE
154 8
196 6
150 4
156 4
169 2
167 2

Fig 4

EXPLANATION OF PLATE LXXIV

Fig 5 Thyroid glands of rabbits (31 to 36) fed on steamed cabbage only Two are goitrous

„ 6 Thyroid glands of rabbits (37 to 42) fed on steamed cabbage and iodine water None are goitrous Compare with Fig 5

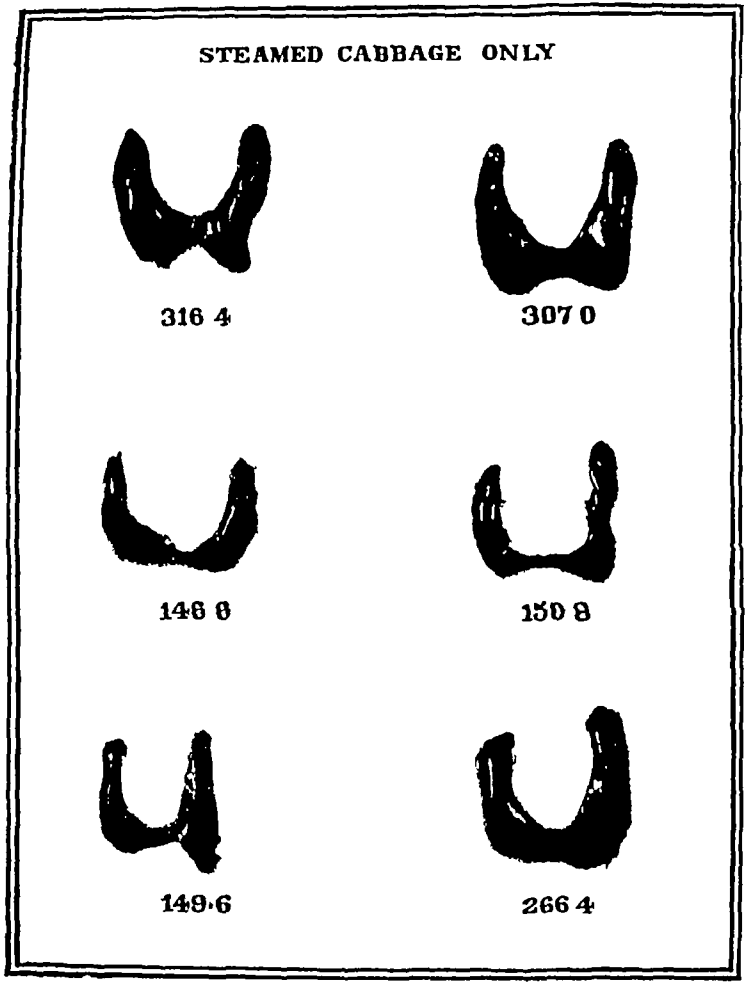

STEAMED CABBAGE ONLY
316 4
307 0
146 8
150 8
149·6
266 4

Fig 5

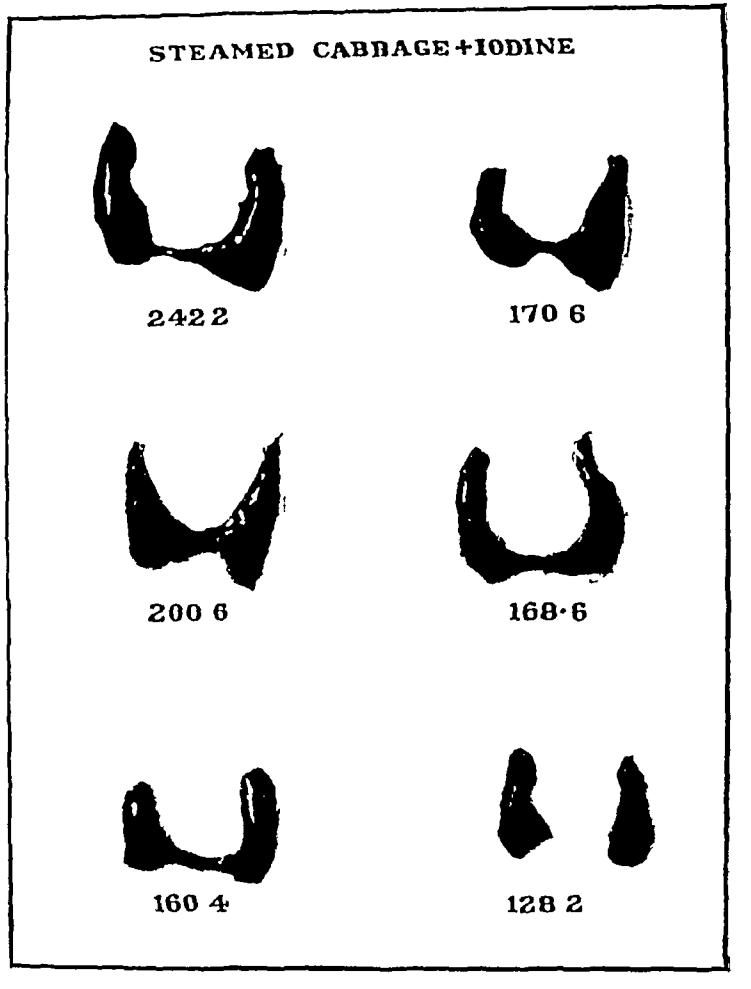

STEAMED CABBAGE+IODINE
242 2
170 6
200 6
168·6
160 4
128 2

Fig 6

EXPLANATION OF PLATE LXXV

Fig 7 Thyroid glands of rabbits (73 to 78) fed on steamed cabbage and carrots Two glands (in the middle row) are slightly enlarged

„ 8 Thyroid glands of rabbits (85 to 90) fed on steamed cabbage and bran Four of these glands are goitrous

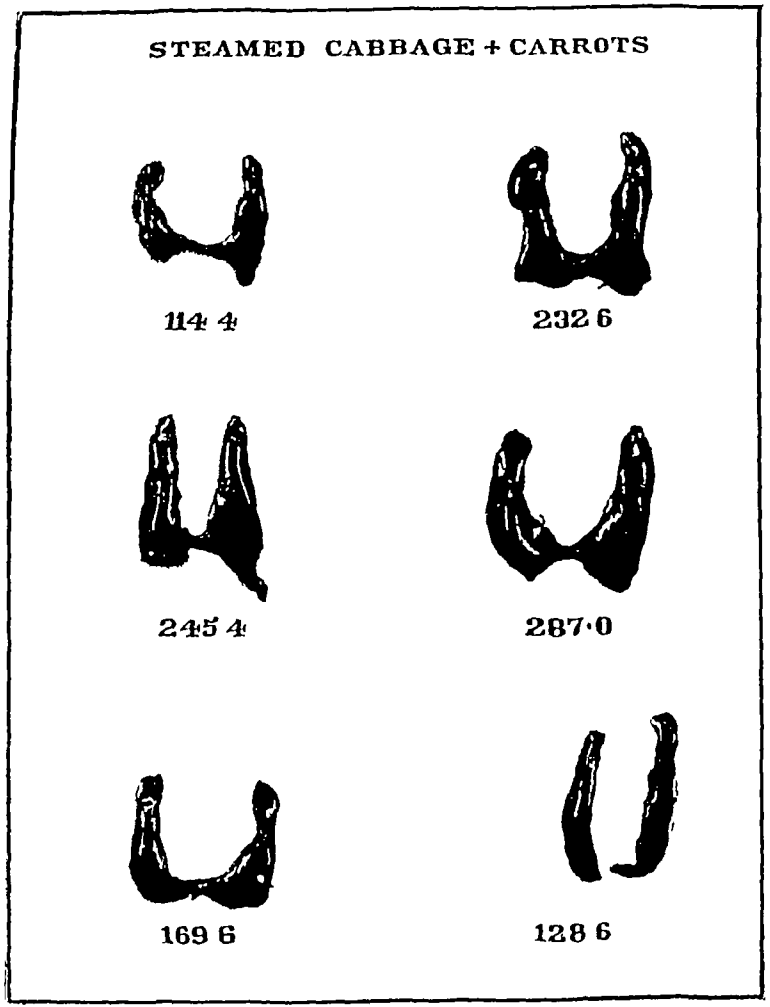

STEAMED CABBAGE + CARROTS
114 4
232 6
245 4
287·0
169 6
128 6

Fig 7

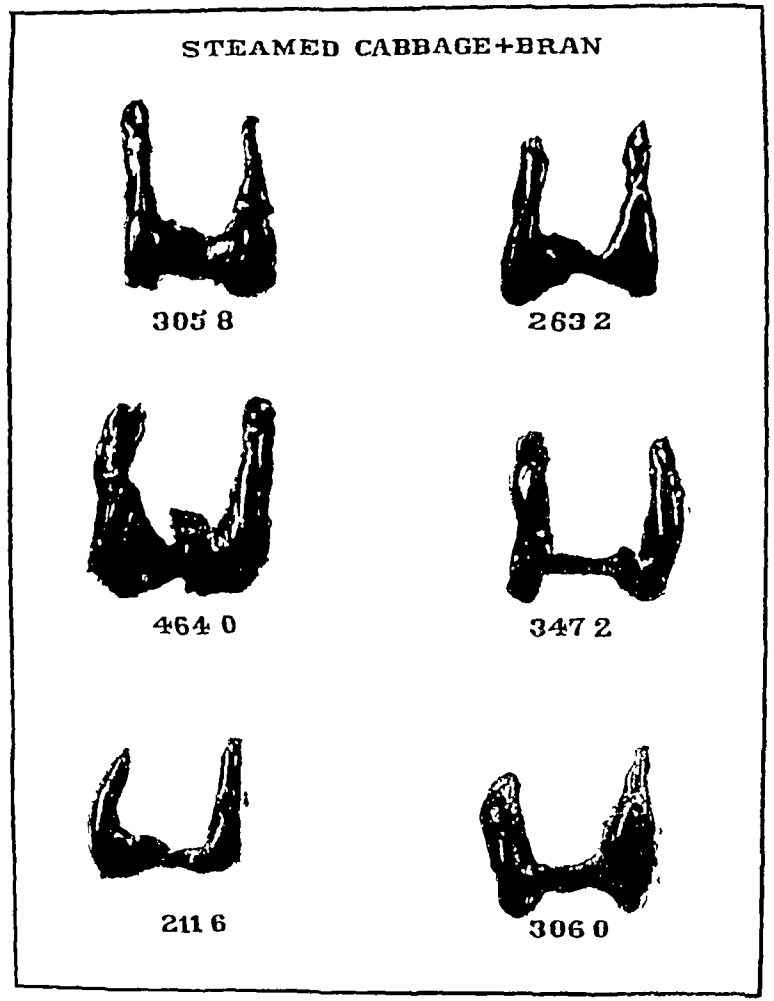

STEAMED CABBAGE+BRAN
305 8
263 2
464 0
347 2
211 6
306 0

Fig 8

EXPLANATION OF PLATE LXXVI

Fig 9 Thyroid glands of rabbits (49 to 54) fed on steamed cabbage which was soaked overnight in fresh chlorine water Two of these glands are slightly enlarged

„ 10 Thyroid glands of rabbits (61 to 66) fed on steamed cabbage which was soaked overnight in a solution of sodium chloride Three of these glands are goitrous

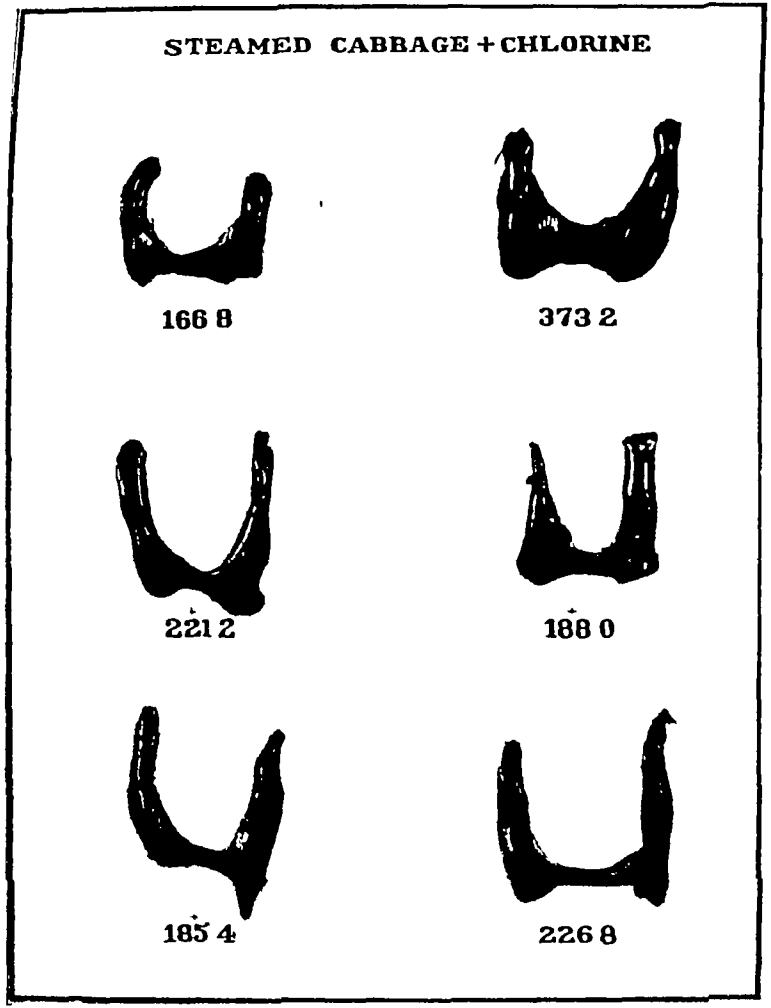

STEAMED CABBAGE + CHLORINE
166 8
373 2
221 2
188 0
185 4
226 8

Fig 9

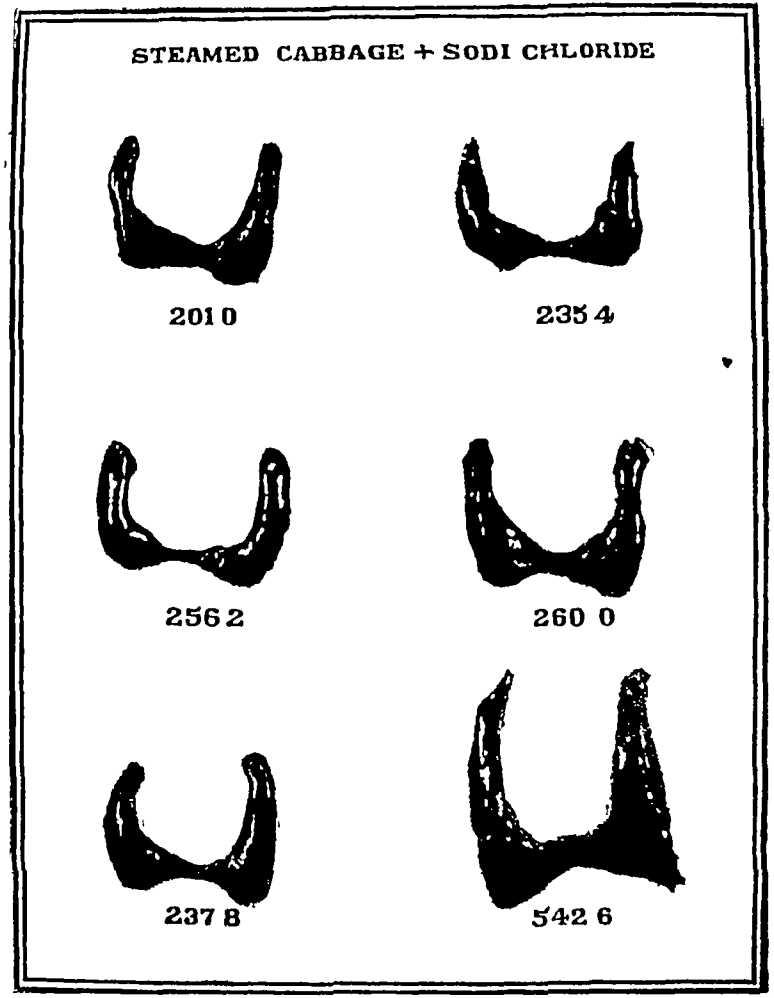

STEAMED CABBAGE + SODI CHLORIDE
201 0
235 4
256 2
260 0
237 8
542 6

Fig 10

Explanation of Plate LXXVII

Fig 11 Thyroid glands of rabbits (67 to 72) fed on steamed cabbage and radiostoleum Five are goitrous

„ 12 Thyroid glands of rabbits (43, 44, 45, 47 and 48) fed on steamed cabbage and thyroxine None are goitrous

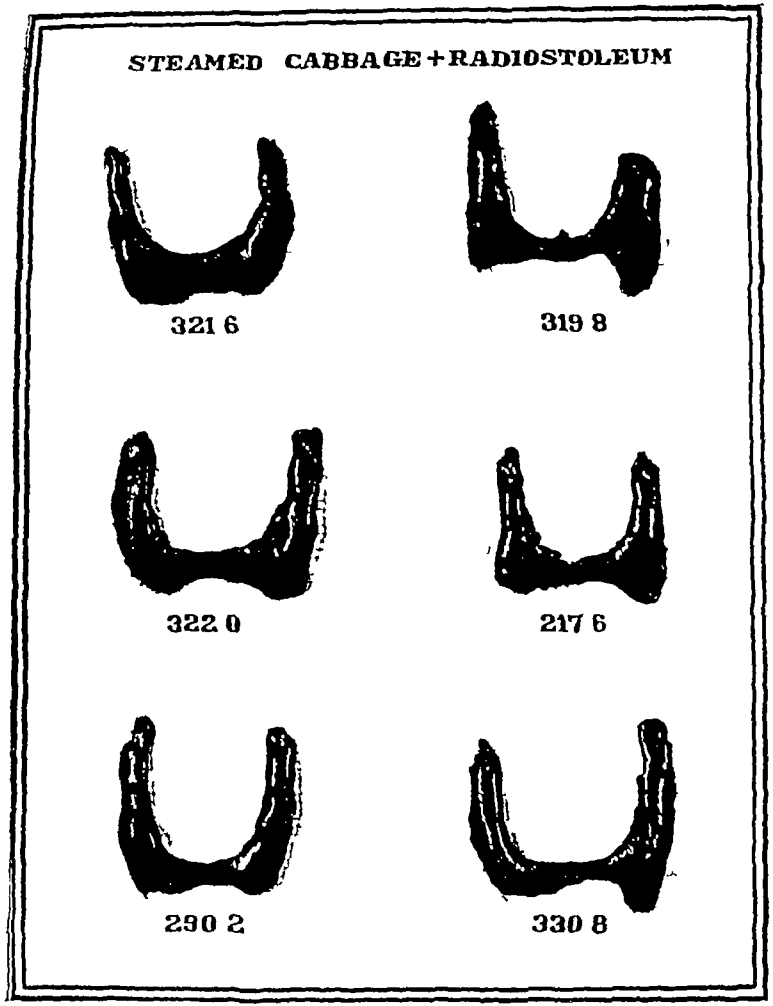

STEAMED CABBAGE + RADIOSTOLEUM
321 6
319 8
322 0
217 6
290 2
330 8

Fig 11

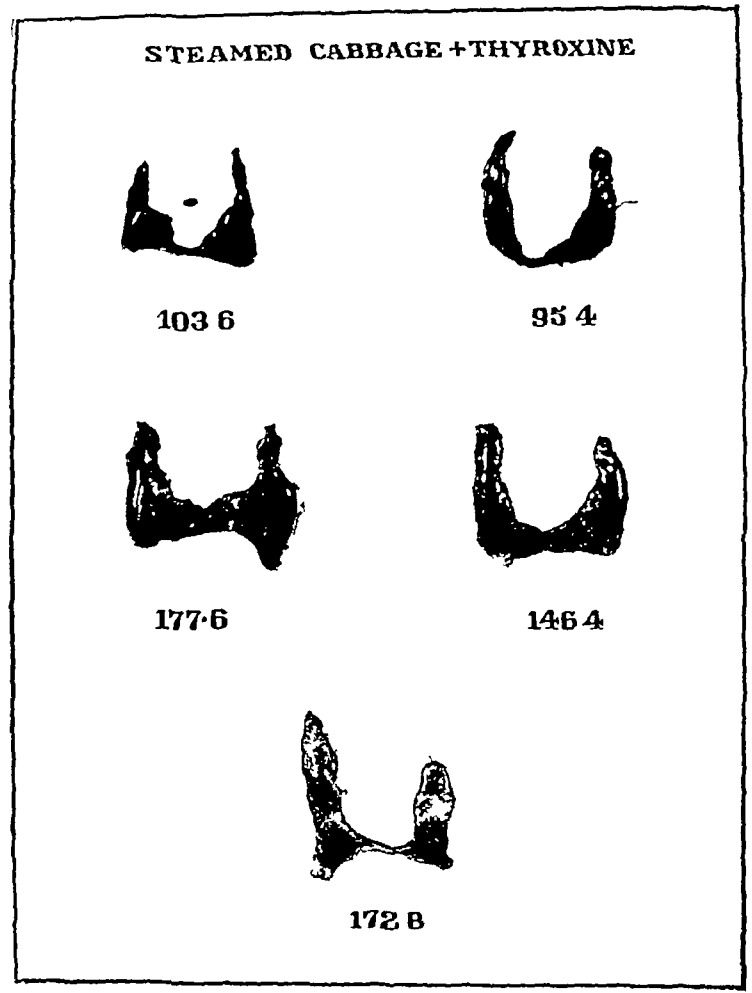

STEAMED CABBAGE + THYROXINE
103 6
95 4
177·6
146 4
172 8

Fig 12

EXPLANATION OF PLATE LXXVIII

Fig 13 Thyroid glands of rabbits (109, 110, 111, 113 and 114) fed on raw cabbage only (3rd experiment) Four are goitrous

„ 14 Thyroid glands of rabbits (116 to 120) fed on raw cabbage (3rd experiment) which was soaked in chlorine water overnight All are goitrous

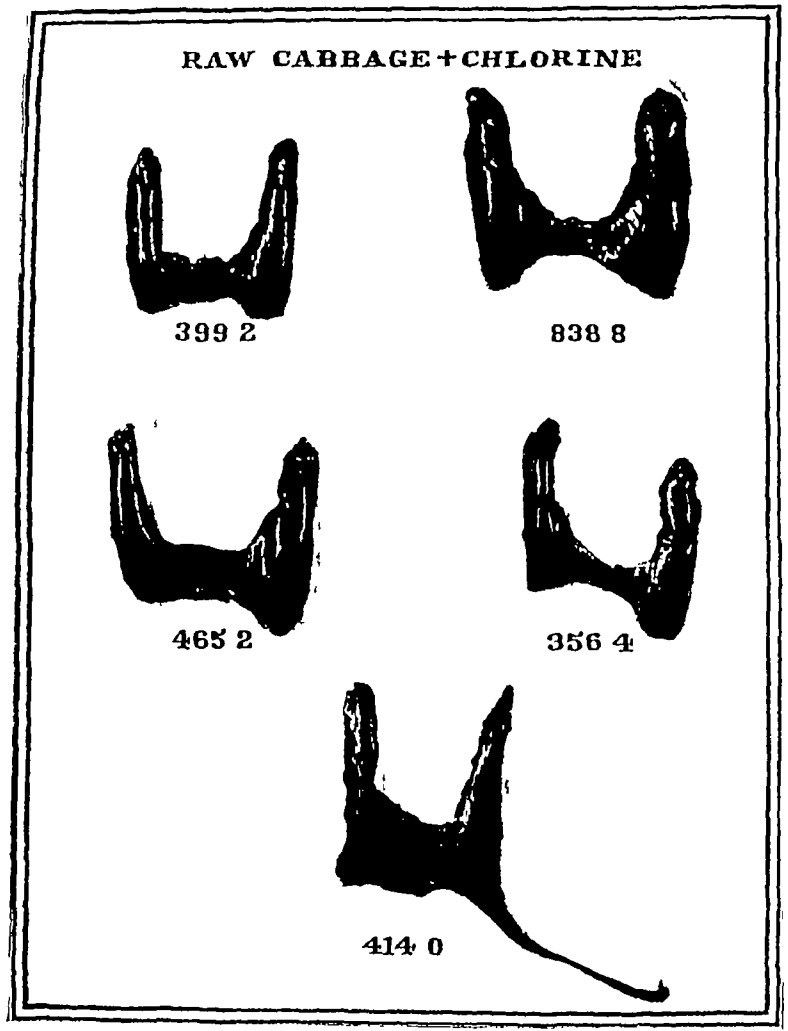

RAW CABBAGE+CHLORINE
399 2
838 8
465 2
356 4
414 0

Fig 13

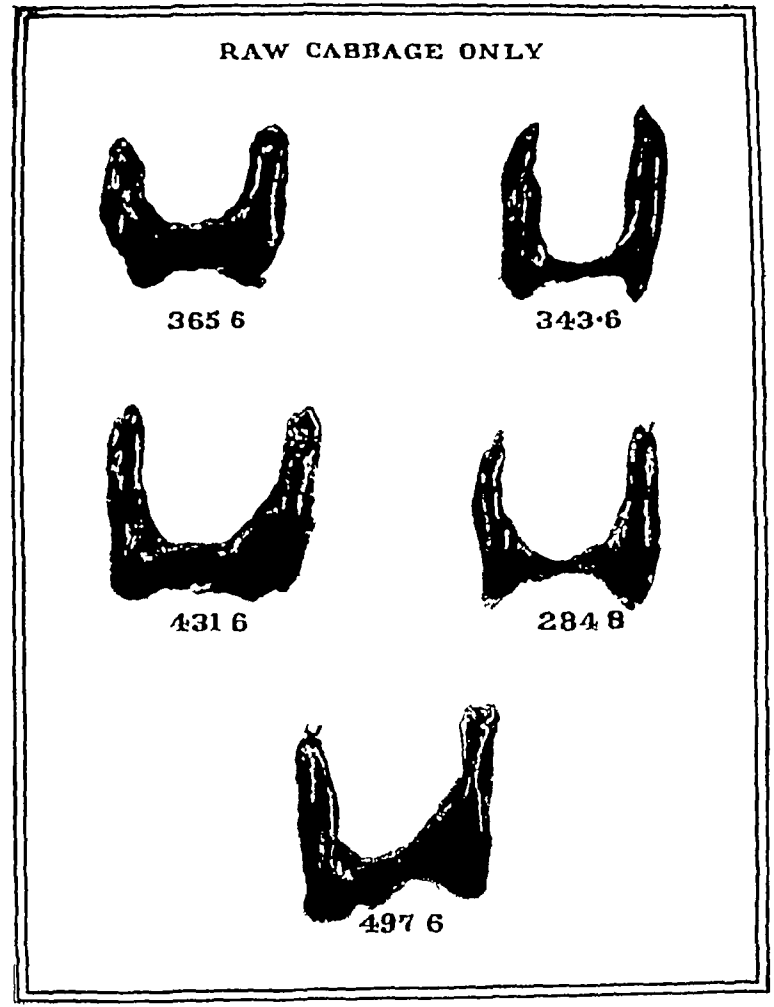

RAW CABBAGE ONLY
365 6
343·6
431 6
284 8
497 6

Fig 14

Explanation of Plate LXXIX

Fig 15 Thyroid glands of rabbits (127 to 132) fed on raw cabbage (3rd experiment) and given 6 grams (approx) of thymol daily One is goitrous Compare with Fig 13

„ 16 Thyroid glands of rabbits (122 to 126) fed on raw cabbage (3rd experiment) and given 2 mg of manganese chloride daily None are goitrous Compare with Fig 13

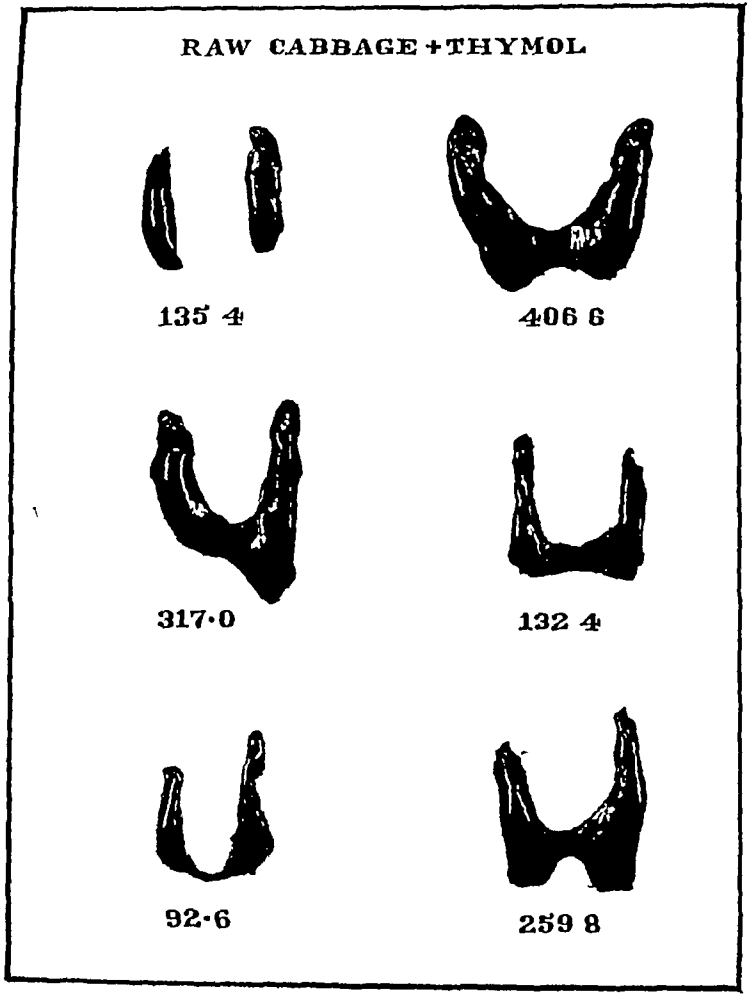

RAW CABBAGE + THYMOL
135 4
406 6
317·0
132 4
92·6
259 8

Fig 15

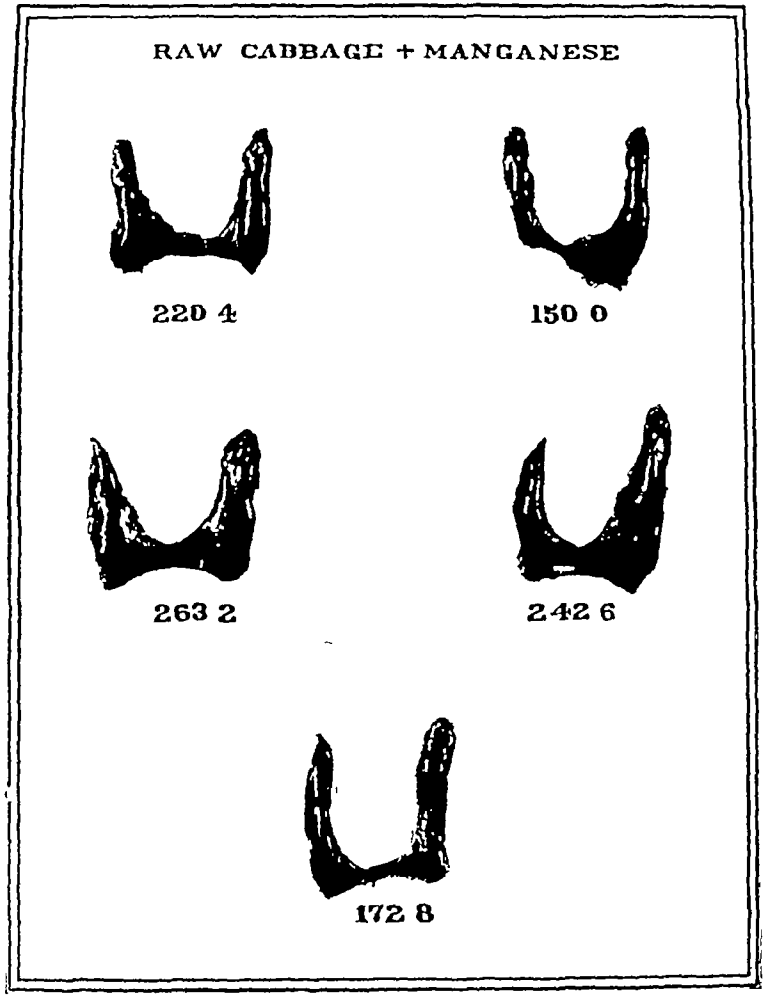

RAW CABBAGE + MANGANESE
220 4
150 0
263 2
242 6
172 8

Fig 16

Explanation of Plate LXXX

Figs 17 and 18 Sections of *non-goitrous* thyroids of rabbits (15 and 18) fed on the control, stock diet (1st experiment) These show the two extremes of histological structure met with in the *non-goitrous* thyroids of stock rabbits in Coonoor

Fig 19 Section of *goitrous* thyroid of rabbit (7) fed on raw cabbage (1st experiment)

„ 20 Section of *goitrous* thyroid of rabbit (4) fed on steamed cabbage (1st experiment) Note great engorgement and absence of colloid

Fig 17

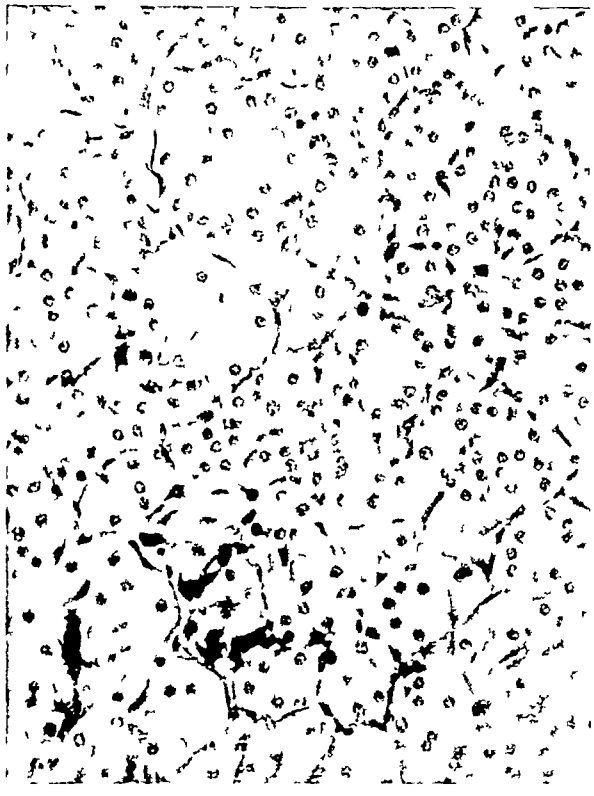
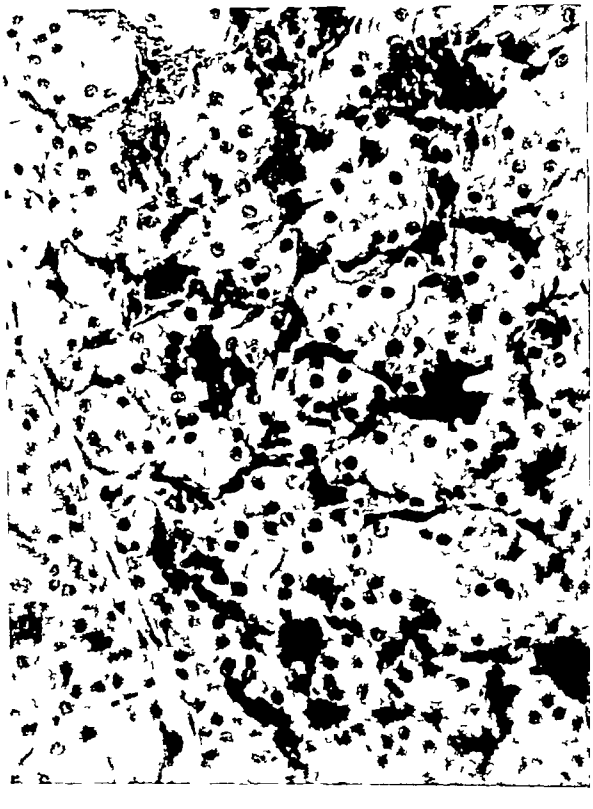


Fig 18



Explanation of Plate LXXXI

Fig 21 Section of *non-goitrous* thyroid of rabbit (58) fed on the control, stock diet used in the second experiment

„ 22 Section of *non-goitrous* thyroid of rabbit (59) fed on the control, stock diet used in the second experiment

„ 23 Section of *goitrous* thyroid of rabbit (32) fed on steamed cabbage 2nd experiment

„ 24 Section of *non-goitrous* thyroid of rabbit (33) fed on steamed cabbage 2nd experiment

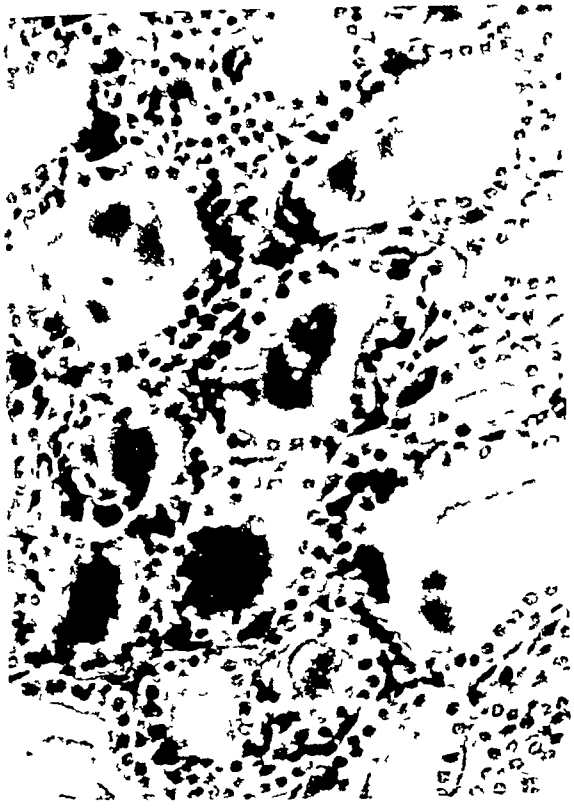
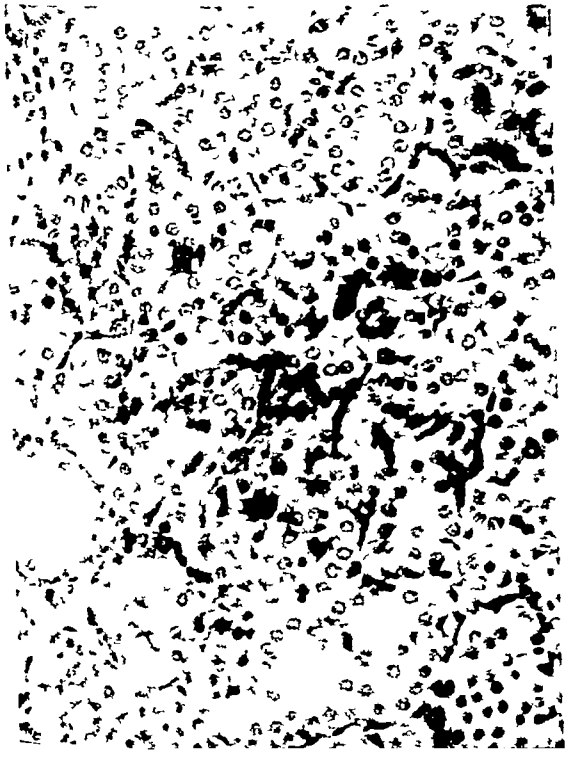


Fig 21

Fig 22



Explanation of Plate LXXXII

Fig 25 Section of *goitrous* thyroid of rabbit (63) fed on steamed cabbage which was soaked in a solution of sodium chloride overnight 2nd experiment

„ 26 Section of *non-goitrous* thyroid of rabbit (52) fed on steamed cabbage which was soaked in chlorine water overnight 2nd experiment Note washed out appearance of the cells

, 27 Section of *goitrous* thyroid of rabbit (71) fed on steamed cabbage (2nd experiment) and given one drop of radiostoleum daily

, 28 Section of *non-goitrous* thyroid of rabbit (113) fed on raw cabbage (3rd experiment) The other five animals in this group were goitrous and their thyroids presented the appearances seen in Figs 19 and 20

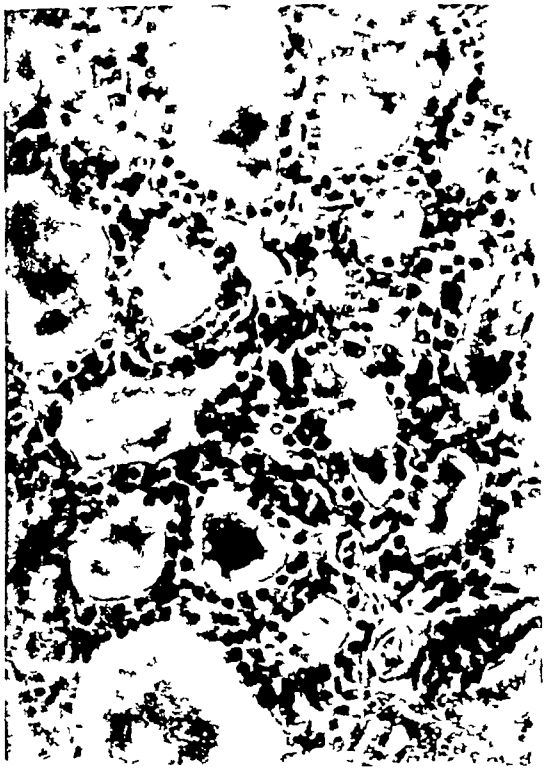


Fig 25

Fig 26

Fig 27

Fig 28

Explanation of Plate LXXXIII

Figs 29 and 30 Sections of *non-goitrous* thyroids of rabbits (26 and 29) fed on raw cabbage and iodine water (2nd experiment) Note that there is no tendency to the accumulation of colloid in the gland, the tendency being in the opposite direction

„ 31 and 32 Sections of *non-goitrous* thyroids of rabbits (47 and 48) fed on steamed cabbage (2nd experiment) and given thyroxine twice a week These represent the two extremes of histological structure seen in animals receiving thyroxine

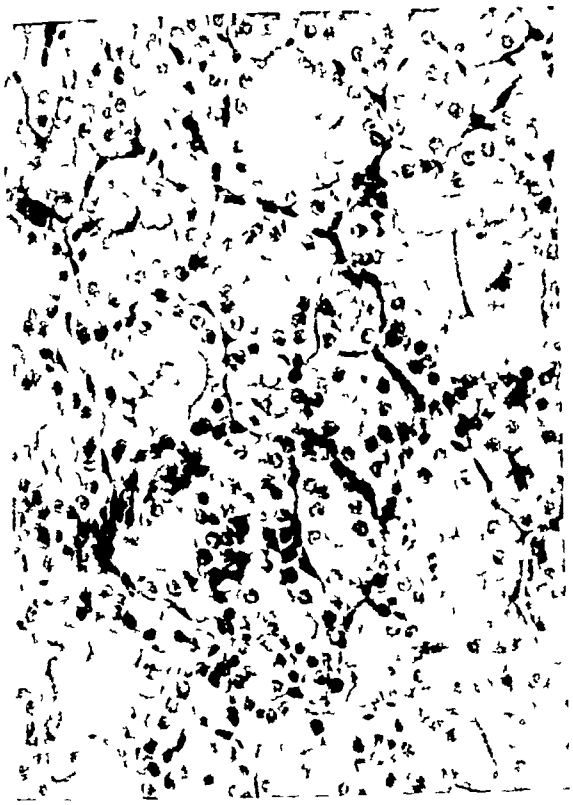


Fig 29

Fig 30

Fig 31

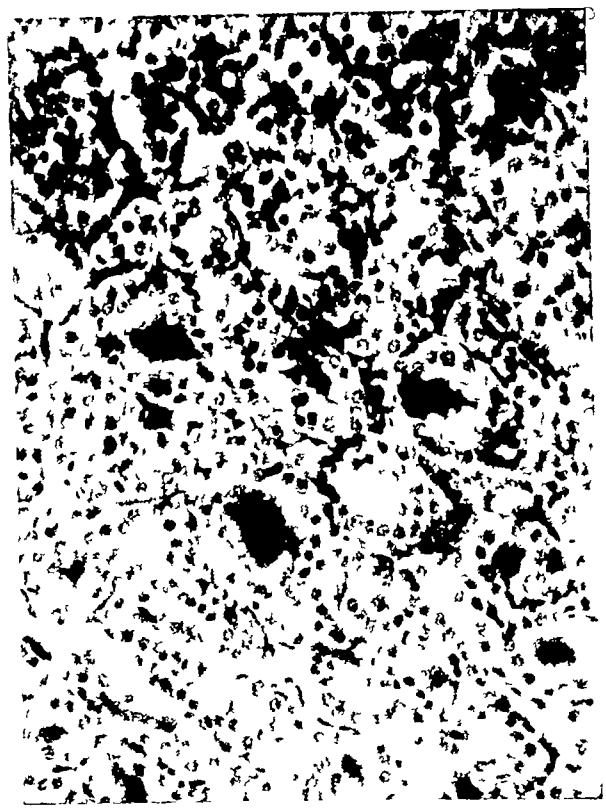


Fig 32

URINARY EXCRETION OF IODINE BY GOITROUS AND NON-GOITROUS PERSONS IN GILGIT

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WITH

A STATISTICAL EXAMINATION OF THE EXPERIMENTAL DATA

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GILGIT, where the senior author's original studies of endemic goitre and cretinism (McCarrison, 1906) were carried out, is situated in the extreme north of Himalayan India and due south of the Little Pamir In 1927 an account was given of the iodine-content of its soils (McCarrison *et al*, 1927) It was found that, while they were invariably low in iodine, goitre was not always endemic, or if endemic not markedly prevalent, in villages whose soils were as low in iodine as those of others wherein endemic goitre and its sequelæ (cretinism, deaf-mutism and idiocy) prevailed with great intensity

The purpose of the present paper is to record the results of an investigation of the iodine-content of the urine of goitrous and non-goitrous persons resident in this locality The published reports of investigations of this kind have usually dealt, not with a comparison of the iodine-metabolism–of which the urinary excretion of iodine is a simple criterion–in goitrous and non-goitrous persons living in the same locality, but with that of persons living in normal and in goitre areas It appeared to us that more valuable information might be gained by contrasting goitrous and non-goitrous residents of the same locality with one another, for, if the level of iodine-metabolism be low in

both, as it is in Gilgit, or high in both as it is in Danzig (Lick, 1927) then there must be some factor or factors other than iodine which determine the occurrence of goitre in some residents while others remain free from it

Material

The samples of urine were obtained with the co-operation of Major J C Pyper, I M S, Agency Surgeon, Gilgit, and his staff, to whom we are much indebted They were collected during the late spring and early summer months, and it must here be emphasized that our results deal only with that season

The samples were sent to Coonoor by post, each being contained in a 6-ounce bottle to which 1 5 grammes of potassium carbonate had been added The journey by post from Gilgit to Coonoor takes approximately 23 days Our first concern was, therefore, to determine whether any loss of iodine occurred in the samples during transit To this end three specimens of urine, obtained from members of the Laboratory Staff, were treated in a way similar to that of the samples from Gilgit they were put up in bottles containing the same amount of potassium carbonate and allowed to stand for 23 days, then iodine-content was then determined at intervals during this period The following were the results —

	Date of examination	Age of urine days	Volume of urine c c	Iodine found γ	Iodine per litre γ
Sample G S—	30-6-30	1	100	7 0	70
	12-7-30	13	25	1·5	60
	22-7-30	23	25	1 75	70
Sample S S—	30-6-30	1	100	7·0	70
	12-7-30	13	25	1 8	72
	22-7-30	23	25	1 75	70
Sample S I—	14-7-30	1	50	1 5	30
	14-7-30	1	50	1 3	26
	14-7-30	1	50	1·5	30
	22-7-30	9	50	1·3	26
	28-7-30	14	50	1 5	30
	28-7-30	14	50	1 3	26
	28-7-30	14	50	1 3	26
	31-7-30	18	50	1 3	26

From these results it is seen that the urinary excretion of iodine by three normal persons in Coonoor, where true endemic goitre is conspicuous by its absence, lay between 26 and 72 γ per litre The results also show that no loss of iodine occurs in urine standing in contact with potassium carbonate for 23 days It may, therefore, be safely concluded that the Gilgit urines did not lose iodine in their transit to Coonoor

Method of Iodine-Determination

Von Fellenberg's colorimetric method of iodine-estimation was employed It was controlled by adding various amounts of iodine to normal urines, and then estimating their iodine-contents The results of these tests were as follows —

Date	Volume of urine c c	Added iodine γ	Iodine found γ	Iodine recovered γ	Per cent recovered γ
		Urine of I C			
1-8-30	50	Nil	5·0	Nil	Nil
	50	1·0	5·8	0·8	80
	50	1·0	6·0	1·0	100
		Urine of S S			
2-8-30	50	Nil	3·0	Nil	Nil
	50	1·0	4·0	1·0	100
	50	1·0	3·8	0·8	80
		Urine of S I			
31-7-30	50	Nil	1·3	Nil	Nil
	50	1·0	2·0	0·7	70
	50	1·5	2·5	1·2	80

In view of the minute quantities of iodine that had to be estimated the amount of added iodine recovered can be said to be satisfactory

It will be seen from Tables I and II that the Gilgit urines, whether from non-goitrous or from goitrous persons, were in general very low in iodine, in consequence, most of the 6-ounce samples had to be used for the first estimation and little remained over for a second Accordingly, what was left over from urines found to contain from 5 to 8 γ of iodine per litre was pooled, similarly, with those containing less than 5 γ per litre Estimations of the

iodine-content of the pooled samples were then made as well as of the amount of added iodine recoverable from them The following were the results —

1 Fifty c c urine gave 0 2 γ iodine, there ought to have been 0 3 γ.
2 Fifty c c urine gave 0 3 γ iodine, there ought to have been 0 3 γ
3 Fifty c c plus 1 0 γ added iodine gave 1 0 γ there ought to have been 1 25 γ, amount of added iodine recovered was 75 per cent
4 Fifty c c plus 0 0 γ iodine added gave 0 3 γ there ought to have been 0 3 γ.
5 Fifty c c plus 1 0 γ iodine added gave 1 0 γ there ought to have been 1 3 γ, amount of added iodine recovered was 70 per cent

It is thus seen that, within the error of estimation, duplicate analyses agreed, and the recovery of added iodine was quite satisfactory

TABLE I

Iodine-content of the urine of goitrous persons in Gilgit

Number	Sex	Age	Urine taken for estimation c c	Iodine found γ	Iodine per litre γ
1	M	40	100	0·6	6·0
2	M	13	62	0·4	6·3
3	M	44	100	0·6	6 0
4	M	16	100	0·8	8·0
5	M	18	100	0 6	6 0
6	M	18	100	0 6	6 0
7	M	23	100	0 6	6 0
8	M	22	100	1 0	10·0
9	M	12	100	0 6	6·0
10	M	9	100	0·1	4 0
11	M	12	100	0·6	6 0
12	M	10	100	0·4	4 0
13	M	10	100	0·8	8·0
14	M	5	100	0·4	4 0
15	M	13	100	0·4	4 0
16	M	5	82	0·6	7·3
17	M	4	100	0 4	4·0
18	M	24	64	0 4	6·3

TABLE I—*concld*

Iodine-content of the urine of goitrous persons in Gilgit

Number	Sex	Age	Urine taken for estimation c c	Iodine found γ	Iodine per litre γ
19	M	36	75	2·0	27·0
20	M	45	100	0·4	4·0
21	F	22	100	0·6	6·0
22	F	22	100	0·8	8·0
23	F	50	100	0·6	6·0
24	F	60	97	1·0	10·0
25	F	28	100	0·6	6·0
26	F	43	100	0·6	6·0
27	F	35	100	0·6	6·0
28	F	11	100	0·6	6·0
29	F	8	100	0·8	8·0
30	F	5	100	0·6	6·0
31	F	10	100	0·6	6·0
32	F	10	100	0·4	4·0
33	F	11	100	0·4	4·0
34	F	16	100	0·8	8·0
35	F	30	40	0·8	18·0
36	F	5	60	0·6	10·0

TABLE II

Iodine-content of the urine of non-goitrous persons in Gilgit

Number	Sex	Age	Urine taken for estimation c c	Iodine found γ	Iodine per litre γ
1	M	21	85	0·6	7·1
2	M	22	100	0·6	6·0
3	M	22	68	0·4	5·9
4	M	9	83	0·4	4·8
5	M	10	100	0·6	6·0

TABLE II—*concld*

Iodine-content of the urine of non-goitrous persons in Gilgit

Number	Sex	Age	Urine taken for estimation c c	Iodine found γ	Iodine per litre γ
6	M	10	100	0·6	6 0
7	M	50	55	0·3	5 5
8	M	20	86	0·8	9·3
9	M	50	55	1·2	22·0
10	M	1	51	0·5	10·0
11	M	5	92	0·6	6·5
12	M	5	90	0·6	6·6
13	M	16	100	0·1	4 0
14	M	19	100	0·6	6 0
15	M	40	100	0·6	6·0
16	M	35	43	1·5	34 0
17	M	45	91	0·5	5 5
18	M	11	100	0·3	3·0
19	F	17	93	1 0	11 0
20	F	18	78	1 2	15 0
21	F	20	76	0·1	5 3
22	F	35	78	0 6	7 7
23	F	27	100	1 2	12·0
24	F	16	77	0·8	10·0
25	F	34	100	0·6	6·0
26	F	42	100	1 8	18·0
27	F	6	92	0·4	4 4
28	F	4	89	1 2	13 5
29	F	5	50	0 2	4 0
30	F	4	97	1 0	10 0
31	F	7	100	0·8	8·0
32	F	26	86	1·0	12·0
33	F	4	94	2·0	22·0

Results of the Analyses

The results of the analyses are set out in Tables I and II together with the age and sex of the subjects from whom the urines were obtained Altogether 69 specimens were examined, of which 36 were from goitrous and 33 from non-goitrous persons Amongst the goitrous subjects there were 20 males aged between 4 and 45 years and 16 females aged between 5 and 60 years Amongst the non-goitrous subjects there were 18 males aged between 4 and 50 years and 15 females aged between 4 and 42 years

It will be noted from a study of Tables I and II that the urinary excretion of iodine both by goitrous and by non-goitrous residents in Gilgit is in general very low during the late spring and early summer months, an observation in conformity with the unusually low iodine-content of the Gilgit soils (McCarrison, *et al* 1927) At this season of the year the iodine-metabolism of the people is in general at a low level, and it is at this season also that goitre is most likely to arise (McCarrison, 1906) It will be observed, however, that although the urinary excretion of iodine by goitrous individuals may be as low as 4 to 6 γ per litre it may be at an equally low level in other individuals who are not goitrous Further, it may be as high as 27 γ in a goitrous individual and yet be lower than this figure in 97 per cent of the non-goitrous persons examined

Before dealing with the significance of the differences in the urinary excretion of iodine in goitrous and non-goitrous subjects it will be of interest to compare the results set out in Tables I and II with those reported from other parts of the world This comparison is afforded in Table III

An examination of this Table shows that the urinary excretion of iodine by persons resident in goitrous localities in Switzerland is half as much again as by non-goitrous persons in Gilgit, in New Zealand it is twice as much, in Norway four times as much, while in Danzig it is 25 times as much In short 'goitre' may occur both in regions where the level of iodine-metabolism of the inhabitants is low as well as in others where it is relatively high, whether or not it is the same type of 'goitre' is another matter It will be noted also that in some non-goitrous persons in New Zealand the urinary excretion of iodine may be as low as in some others who are goitrous, while it may be higher in the latter than in all non-goitrous persons in Gilgit as well as in certain non-goitrous persons in Switzerland and Italy It is obvious, therefore, that low levels of iodine-metabolism–even very low levels–are not always associated with goitre, while high levels do not always signify freedom from it If, therefore, iodine-deficiency is to be regarded as an essential cause of goitre, the degree of deficiency necessary for its production varies greatly in different parts of the world and in different individuals in the same locality, further, the deficiency must be relative to other factors which are the true goitrogenic agents In our experience in animals a urinary excretion of iodine of such relatively high range as 75 (New Zealand) to 140 γ (Norway) per litre (Table IV) is incompatible with the occurrence of pure *hyperplastic goitre* in

TABLE III

Showing the daily urinary excretion of iodine (in γ)

Locality	Number of persons	Normal area		Goitre area	
		Range	Average	Range	Average
Switzerland	7	53 to 108	61		
	11			4 to 29	19
	12			5 to 28	17
Italy	8	30 to 140	72		
Norway	5		173		
	53			6 to 140	53·7
New Zealand	35*	11 to 381	19		
	27†			5 to 75	25·0
Danzig	5			200 to 590	343·0
Gilgit	33	(non-goitrous persons)		4·5 to 51	13·7‡
	36	(goitrous persons)		6 to 40·5	10·8‡

* Non-goitrous persons

† Goitrous persons

‡ Calculated from the iodine-content per litre (Tables I and II) on the assumption that the average individual excretes 1·5 litres of urine per day

rabbits though not incompatible with the occurrence of *lymph-adenoid goitre* in rats, the latter type of goitre not being related in its origin to iodine-deficiency (McCarrison, 1927)

Significance of the Differences Revealed by the Analyses.

It has been noted from Tables I and II that while the urinary excretion of iodine by people in Gilgit is in general very low, it is somewhat lower in goitrous than in non-goitrous persons It remains now to consider the significance of this difference

The data, set out in Tables I and II, were submitted to Professor K B Madhava, for statistical scrutiny The results of his examination are summarized in Table IV, wherein he has contrasted thirteen pairs of universes

It will be observed from Table IV that although in all contrasted universes, except the pair numbered II, goitrous individuals excrete less iodine than non-goitrous, the difference is in no case definitely significant Even in the universes numbered respectively IX and X the significance is 'doubtful' in the one and 'very doubtful' in the other, Professor Madhava regarding the difference in

TABLE IV

Showing the significance of the differences in mean value of γ in the universes contrasted

Universes contrasted		Mean γ in (1) minus mean γ in (2)	P (Per cent)	Significance
(1)	(2)	(3)	(4)	(5)
I Goitrous males aged 1 to 10	Non-goitrous males aged 1 to 10	— 1·4333	20	Not significant
II Goitrous males aged 11 to 20	Non-goitrous males aged 11 to 20	+ 0·4250	80	Do
III Goitrous males aged 21 to 40	Non-goitrous males aged 21 to 40	— 0·7400	90	Do
IV Goitrous males aged 41 to 50	Non-goitrous males aged 41 to 50	— 5·5472	35	Do
V Goitrous females aged 1 to 10	Non-goitrous females aged 1 to 10	— 3·5167	25	Do
VI Goitrous females aged 11 to 30	Non-goitrous females aged 11 to 30	— 2·8404	20	Do
VII Goitrous females aged 31 to 50	Non-goitrous females aged 31 to 50	— 3·5667	40	Do
VIII Goitrous males	Non-goitrous males	— 1·6217	45	Do
IX Goitrous females	Non-goitrous females	— 3·1995	5	Doubtful more probably not significant
X Goitrous both sexes	Non-goitrous both sexes	— 2·3435	8·1	Very doubtful most probably not significant
XI Goitrous males	Goitrous females	— 0·4488	75	NOT SIGNIFICANT
XII Non-goitrous males	Non-goitrous females	— 2·0267	36	Do
XIII Males	Females	— 1·4919	24	Do

the former pair (IX) as 'more probably not significant' and that in the latter as 'most probably not significant' His conclusion is as follows —

> 'While the data reveal no incontrovertible association between the occurrence of goitre and the urinary excretion of iodine, it is possible that such association may exist in females (IX and X, Table IV), this possibility is, however, remote But if it does exist it is femininity that determines it, there being definitely no such association in males.'

Here we may draw attention to three points which appear to us to be of importance in connexion with investigations of this kind. (1) Comparison should be instituted not between persons living in 'normal' and in 'goitre' areas, but between goitrous and non-goitrous persons living in the same goitre area. (2) Care has to be taken that the subjects from whom the samples are obtained have not had access to the household remedy iodine. In our own series there were two which yielded surprisingly high figures, on further inquiry these were found to have been obtained from non-goitrous women who had iodine applications to wounds on their legs, the inclusion of these two samples would have vitiated our results. (3) The number of urines examined should be as large as possible and the results should be submitted to statistical scrutiny, otherwise conclusions may be drawn which are not justifiable. Thus in almost all contrasted universes in our series (Table IV) goitrous individuals excreted less iodine in the urine than non-goitrous, but in no instance was this difference found, on statistical scrutiny, to be definitely significant.

Diet.

It is obvious from the amount of iodine excreted in the urine that the diet of the Gilgitis whose urine was examined was very low in this substance. Each sample submitted to us had an accompanying note stating the general composition of the diet of the person from whom the sample was obtained. The food was not essentially different in goitrous and in non-goitrous subjects. It was wholly vegetarian in all cases but three, two of the three included meat in their dietaries, the third included milk in his. The basis of the diet was maize and wheat, only 3 out of the 69 used barley and only one used rice. Six used legumes and of these 3 were goitrous and 3 were not. Vegetables are sparsely grown in Gilgit and were sparely eaten by the subjects of this inquiry. Their drinking-water was that flowing through the irrigation channels, it is grossly polluted.

This dietary has three conspicuous faults: (1) lack of suitable protein, (2) deficiency of fat-soluble vitamins and of vitamin C, and, (3) deficiency of iodine. This combination of faults—as shown in a recent paper from this laboratory (McCarrison, 1930)—is definitely favourable to the development of goitres of a degenerative type. The functional efficiency of the thyroid gland appears to depend in considerable measure on the adequate provision of fat-soluble vitamins in the diet (McCarrison, 1930). But since the food eaten by the non-goitrous subjects appeared to be as faulty in these regards as that eaten by goitrous persons, the conclusion would seem to be justified that some agent other than dietetic ones was responsible for the occurrence of the disease.

Conclusions.

1. The urinary excretion of iodine by non-goitrous as well as by goitrous persons resident in Gilgit is, in general, very low during the late spring and

early summer months This observation is in conformity with the low iodine-content of the Gilgit soils

2 The data reveal no incontrovertible association between the urinary excretion of iodine and the occurrence of goitre It is possible, though the possibility is remote, that such association may be present in females, there is definitely none in males

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THE MECHANISM OF INFECTION WITH MALARIA IN CHILDREN LIVING UNDER ENDEMIC AND HYPERENDEMIC CONDITIONS

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INTRODUCTION

DURING a recent malaria survey of some tea gardens near Mariani in the Sibsagar district of Assam the opportunity was taken to measure the spleens of a large number of children by Christophers' (1924) method, and to take blood films from as many children as possible, using Sinton's (1924) fowl cell dilution method to count the number of parasites present, in the hope of throwing some light on the relation between the spleen and parasite rates, on the cause of the variations in the size of the spleen, and on allied problems

The district in which the survey was made is some ten to twenty miles south of Jorhat, near the foot of the Naga Hills and about 350 feet above sea level, the climate is extremely moist, the average annual rainfall being 82 inches, which falls chiefly between April and October, coinciding with the hot weather, during the whole of which transmission of malaria is possible

In order to avoid confusion of the results, all children not born on the estate on which they were examined and who had been resident for less than two years have been omitted from all tables in this paper, those not born on the estate but who had been resident for more than two years have had their age counted from the date of their arrival on the estate in all age analyses Their numbers are small, so that even if they are thus put into an age group with which they are not strictly comparable, the error introduced will be slight

Of the total 338 infections found, 241, or 71 per cent were with *P falciparum*, 9 or 3 per cent with mixed *P falciparum* and *P vivax*, 68 or 20 per cent with *P vivax* and 20 or 6 per cent with *P malariæ* As the majority of infections were with one species of parasite, and the others showed no special distribution as regards age or association with enlarged spleens, and as it is impossible to distinguish between enlargement of the spleen due to one species of parasite from that due to another, the species of parasite has been ignored in this paper and 'infections' only considered

The varying severity of malaria in these lines depends on the varying proximity of the breeding places of the dangerous anophelines, and not on climatic differences altering the length of the transmission period It is therefore thought that they are eminently suitable for comparison with one another and that any deductions drawn from them may be applied to areas in which there is an equally long transmission period, and in which the malaria is more or less static it is probable that some modification might be needed before they could be applied to widely differing areas such as the Punjab, in which there are rare and very short periods of intensive malaria, followed by long periods of quiescence

The relation between the spleen and parasite rates

In Table II the spleen and parasite rates of the different series are set out, the percentages given here differ slightly from those given elsewhere because in calculating the spleen rate only those children in whom the blood was examined were included

Table II

Showing the relation between the spleen and parasite rates

Series	Spleen rate Per cent	Parasite rate Per cent	Parasite rate amongst those with enlarged spleens Per cent	Parasite rate amongst those with negative spleens Per cent
Over 75 per cent	83	56	58	52
50–75 „	65	47	51	38
25–50 „	40	42	58	32
Under 25 „	14	16	55	10

It will be noted that the relation between the two rates is much the same as is usually found, and in particular that (1) in the areas with a high spleen rate the parasite rate is lower than the spleen rate, while in areas with a low spleen rate the parasite rate is as high as or higher than the spleen rate; (2) the parasite rate among those with enlarged spleens remains practically constant between 50 and 60 per cent, (3) the parasite rate among those without enlarged spleens shows marked variations, varying directly with the spleen rate

The explanation of the relation between these two rates, and particularly of the points set out above has never been clearly given The presence of infection, as indicated by the parasite rate, being the direct cause of the spleen rate, one would expect some direct relation to exist between them, the parasite rate being either constantly higher than the spleen rate, or at any rate maintaining a constant relation to it

Schuffner (1920) believes that such a constant relation exists, and that the great majority of children infected with malaria have an enlarged spleen, which can be palpated if the examiner is sufficiently careful, as it by no means always reaches the costal margin It has been pointed out by Christophers (1929) and has been noted by every one taking careful measurements of a large series of spleens, that the enlarged spleen has a definite modal size which, however low the spleen rate, invariably lies some 3 cm or more below the costal margin If, then, Schuffner's explanation is correct, there must be a second modal distribution, some centimetres above the costal margin, and entirely distinct from the normal mode This may explain the relation between the spleen and the parasite rates, but it leaves unexplained the behaviour of those spleens which project below the costal margin, centre round the usual mode, and form what is usually known as the spleen rate In addition to this main explanation Schüffner also postulates that cases of parasitæmia may occur without enlargement of the spleen in very early cases, in cases of acute cachexia, and after the use of quinine, although these doubtless do occur they will be insufficient in number in such an area as that here dealt with to affect the total result to any marked degree

In trying to find an explanation of this problem two points deserve consideration, firstly, on examination of a series of blood films, even by the thick film method, only some 60 per cent of infections are discovered The author, working in an equally endemic area (West Africa), has made three series of 'intensive examinations,' taking a number of children, examining their spleens, temperatures, and bloods, and re-examining those found to have negative blood films on each of the next six days The results of these examinations, set out in Table III, show that only 55 to 70 per cent of the infections eventually found were discovered on the first examination, although series 2 and 3 were examined by the thick film method

It is reasonable to assume that in areas of static endemicity such as that in which the Assam series of examinations were made, all those children with

TABLE III

Showing the results of seven successive examinations of three series of West African children

Series	Spleen rate Per cent	Parasite rate on first examination Per cent	Parasite rate after all examinations Per cent	Percentage of parasites found on on first examination
Intensive 1	48	48	85	56
„ 2	56	48	80	59
„ 3	78	67	94	71

enlarged spleens are infected with malaria, and as in these series the parasite rate in those with enlarged spleens remains practically constant at about 55 per cent, it is probable that this serves as a criterion of the proportion of infections which are being found at a single examination In the West African intensive series the two values are very close to one another, the actual figures being set out in Table IV

TABLE IV

Showing the relation between the parasite rate in those with enlarged spleens, and the percentage of infections found at a single examination

Series	Parasite rate in those with enlarged spleens Per cent	Percentage of parasites found on first examination
Intensive 1	63	56
„ 2	53	59
„ 3	73	71

From this evidence it may be concluded that only a fraction of the actual infections are found on a single examination, and that the parasite rate among those with enlarged spleens is a useful criterion of the percentage of infections which are being discovered Therefore to obtain an idea of the true infection rate the parasite rate should be multiplied by a factor based on this If this is done an infection rate is found which is, of course, higher than the spleen rate, but still bears no constant relationship to it

The second important point bearing on this matter is the method of production of a parasite rate, or spleen rate, this was elaborated by Christophers (1915) in connection with his *splen* theory, who pointed out that if 100 infections were spread completely at random amongst 100 people, it is extremely unlikely that each person would get a single infection, the probable result being

TABLE V

Showing the 'true infection rate' in the Assam series

Series	Parasite rate (p) Per cent	Parasite rate amongst those with spleens (x) Per cent	True infection rate $(p\frac{100}{x})$ Per cent
Over 75 per cent	56	58	97
50–75 „	47	51	92
25–50 „	42	58	72
Under 25 „	16	55	29

TABLE VI

Showing the infection rate resulting from the distribution of a known number of infections amongst 100 *persons*

Number of infections distributed *n*	Infection rate resulting Per cent	Number of infections distributed *n*	Infection rate resulting Per cent
10	9	170	82
20	18	180	84
30	26	190	85
40	33	200	87
50	39	220	89
60	45	210	91
70	51	260	93
80	55	280	94
90	60	300	95
100	63	320	96
110	67	340	97
120	70	360	97
130	73	380	98
140	76	400	98
150	78	420	98·5
160	80		

that some would escape infection, some would get a single infection, some would be twice infected, a few three times, etc The actual probable distribution follows definite mathematical laws, the number getting 0, 1, 2, 3, etc, infections following the first, second, third, fourth, etc, terms of the binomial expansion of $\frac{\{(N-1)+1\}^n}{N^{n-1}}$ where N equals the number of persons in the population and n equals the number of infections distributed In the example cited, where 100 infections were distributed *completely at random* between 100 persons, 37 would escape infection, 37 would get one infection, 18 would get two, 6 would get three, the distribution of the remainder being uncertain, the infection rate would be 63 per cent Similarly every infection rate, if fairly high, is due to the distribution of a much larger number of infections Table VI, which shows the infection rate produced by the distribution of a given number of infections amongst 100 persons, is a reproduction of a part of one of Christophers' tables

If, now, instead of dealing with the relationship between the spleen and parasite rates, we deal with the relation between the number of infections required to produce the true infection rate (called henceforward 'infection n') and the number of spleens that would have to be distributed to produce the spleen rate (called 'spleen n'), we find that these bear a fairly constant direct linear relation to each other, the latter being about 40 to 50 per cent of the former In Table VII these figures are set out for the Assam series, and also

TABLE VII

Showing the relationship between the number of infections required to produce the true infection rate (infection n), *and the number of spleens to produce the spleen rate (spleen* n)

Series	Spleen rate	Spleen n	Parasite rate in those with spleens (r)	Parasite rate (p)	True infection rate ($p\frac{100}{r}$)	Infection n	Ratio between 'spleen n' and 'infection n'
Assam—							
Over 75 per cent	83	175	58	56	97	350	0·50
50–75 "	65	105	51	47	92	250	0·42
25–50 "	40	50	58	42	72	125	0 40
Under 25 "	14	15	55	16	29	35	0 43
African—							
Intensive 1	48	70	63	48	85 *	190	0·37
" 2	56	80	53	48	80 *	160	0·50
" 3	78	150	73	67	94 *	280	0 53
Freetown 1	50	70	56	41	73	130	0·54
" 2	71	120	75	72	96	320	0·37

* In these cases the true infection rate is calculated by the final parasite rate after seven examinations

for the West African series, to show that the relationship holds good in similar areas elsewhere

The 'spleen n' and the 'infection n' here have a fairly constant relationship to each other, and the constancy of this relation is truly remarkable when one considers the manner in which errors are magnified in this method of reasoning, suppose for instance that in the first example given in this table (series over 75 per cent), the actual parasite rate had been 5 per cent lower than as given in the table, this would have meant a true infection rate 9 per cent below that given above, and an 'infection n' 130 less than the one here calculated, the ratio between the spleen n and the infection n then being 0 83, instead of 0 50

This relationship between the number of infections required to produce the infection rate and the number of spleens which have to be distributed to produce the spleen rate must mean that the dose of sporozoites inoculated by the infecting anophelines varies greatly, producing an infection of varying severity [*see* Christophers, (1910)], and that only in some 40 to 50 per cent of cases is this transmitted infection of sufficient severity to cause marked enlargement of the spleen, the number of persons actually developing infection, and developing an enlarged spleen, is in accordance with the formula given and as set out in Table VI

If we attack this problem from the reverse point of view, and estimate from a given number of infecting bites, distributed among 100 people, the number who will develop infection, the number of people in whom parasites

TABLE VIII

Showing the parasite and spleen rates produced by a given number of infecting bites

Series	1	2	3	4	5
	Number of infecting bites	Actual infection rate	Parasite rate (60 per cent of col 2)	Number of spleens distributed (50 per cent of col 1)	Spleen rate
1	400	98	59	200	87
2	200	87	52	100	63
3	100	63	38	50	39
4	60	45	27	30	26
5	40	33	19	20	18
6	20	18	11	10	9·5

will be found, the parasite rate (on the presumption that we find 60 per cent of all infections), the number of people who will develop enlarged spleens

(on the presumption that 50 per cent of the infections transmitted are of sufficient severity to cause enlargement of the spleen), it will be found that the relation between the spleen and the parasite rates is such as is normally seen This is elaborated in a hypothetical series in Table VIII

In these cases the relation between the spleen rate and the parasite rate is that which is normally found in areas of more or less static endemicity, in those with a high spleen rate the parasite rate is relatively low, in those with a low spleen rate the parasite rate is as high as or higher than it

Taking this same series and investigating the parasite rate in those with and in those without enlarged spleens, we find that again the results are in accord with those usually found in these areas (Table IX)

TABLE IX

Showing the parasite rates in those with, and in those without, enlarged spleens, resulting from a known number of infecting bites

Series	Those with enlarged spleens			Those without enlarged spleens			
	No	Actual infection rate	Parasite rate	No	Actual number infected	Parasites found in 60 per cent of these	Parasite rate
1	87	100	60	13	11	7	51
2	63	100	60	37	23	14	38
3	39	100	60	61	24	14	23
4	26	100	60	74	19	11	15
5	18	100	60	82	15	9	13
6	9	100	60	91	9	5	5

Note—The actual number infected, amongst those without enlarged spleens, is calculated by subtracting the number developing enlarged spleens from the total number infected

The parasite rates amongst those with and without enlarged spleens show the same relation to the spleen rate as is usually found, and was found in the Assam series, in those with enlarged spleens it remains constant, while in those without enlarged spleens it varies directly with the spleen rate

SPLEEN AND PARASITE FINDINGS

Parasite findings in relation to age

In Table X, which shows the parasite rate in the different age groups, the figures have been divided into two yearly age groups in order to ensure that

there are sufficient children in every group to give a reliable result, the figures for the two series with the low spleen rates have been further run together with the same purpose

TABLE X

Showing the percentage of children in each age group who were found to be infected

SERIES	OVER 75 PER CENT	50-75 PER CENT	25-50 PER CENT	UNDER 25 PER CENT
	Percentage positive	Percentage positive	Percentage positive	Percentage positive
0-1-2	68	59	37	7
3-4	61	51		
5-6	55	46	18	33
7-8	49	46	26	19
9-10	50	28		

In the two series with the higher spleen rates the parasite rate appears to decrease gradually but definitely from the commencement, in the two series with the lower spleen rates the parasite rate appears to reach its maximum at the age period 5 to 6 years, and thence to decrease again

The numerical frequency of parasites is set out in Table XI, in which it will be seen that in the lines with spleen rates over 75 per cent, the majority of the counts in the children under three years old are high, over 1,000 per c mm In the lines with spleen rates between 50 and 75 per cent only one child under two years old was examined, consequently the age group 0-1-2 shows no more heavy infections than the later ages In the lines with spleen rates between 25 and 50 per cent the heavy infections appear to be more frequent in the children up to the age of four, from which age they decrease, while the figures for the lines with spleen rates below 25 per cent are too small to allow of any reliable conclusions being drawn

These results are similar to those which have been found by other observers in the past, and are consistent with the theory of a period of acute infestation in early childhood, followed by a stage of immune infestation in later childhood, the only difference between the four areas being that in those with the low found, the rates the period of acute infestation lasts till a later time of all infections of immune infestation is reached

There is no marked difference in the value of the parasite counts in the four series, which would suggest that there is no marked difference in the severity of malaria in them

TABLE XI

Showing the age distribution and numerical values of parasite infestations

Series Spleen rate Per cent	Age	Number with infections			Total
		Under 100 per c.mm	100–1,000 per c.mm	Over 1,000 per c.mm	
Over 75	0–1–2	4	6	13	23
	3–4	5	15	7	27
	5–6	7	26	7	40
	7–8	8	13	4	25
	9–10	3	6	0	9
50–75	0–1–2	5	13	5	23
	3–4	8	24	12	44
	5–6	8	19	10	37
	7–8	2	11	3	16
	9–10	1	3	1	5
25–30	0–1–2	1	2	4	7
	3–4	1	5	6	12
	5–6	7	8	9	24
	7–8	0	4	0	4
	9–10	2	2	0	4
Under 25	0–1–2	0	0	0	0
	3–4	0	2	0	2
	5–6	6	3	4	13
	7–8	2	4	1	7
	9–10	0	0	0	0

Spleen findings in relation to age

(1) *Spleen rate*—This shows a slight increase till it reaches a maximum in children aged three to four in the series with the higher spleen rates, five to six in the 25–50 per cent series, and seven to eight in the under 25 per cent series, after which it shows a steady tendency to decline in the older children

TABLE XII

Showing the spleen rates in the different age groups

SERIES Spleen rate Per cent	AGE				
	0–1–2	3–4	5–6	7–8	9–10
Over 75	76	90	78	80	68
50–75	60	76	58	57	51
25–50	30	47	49	35	37 5
Under 25	7	15	21	24	12

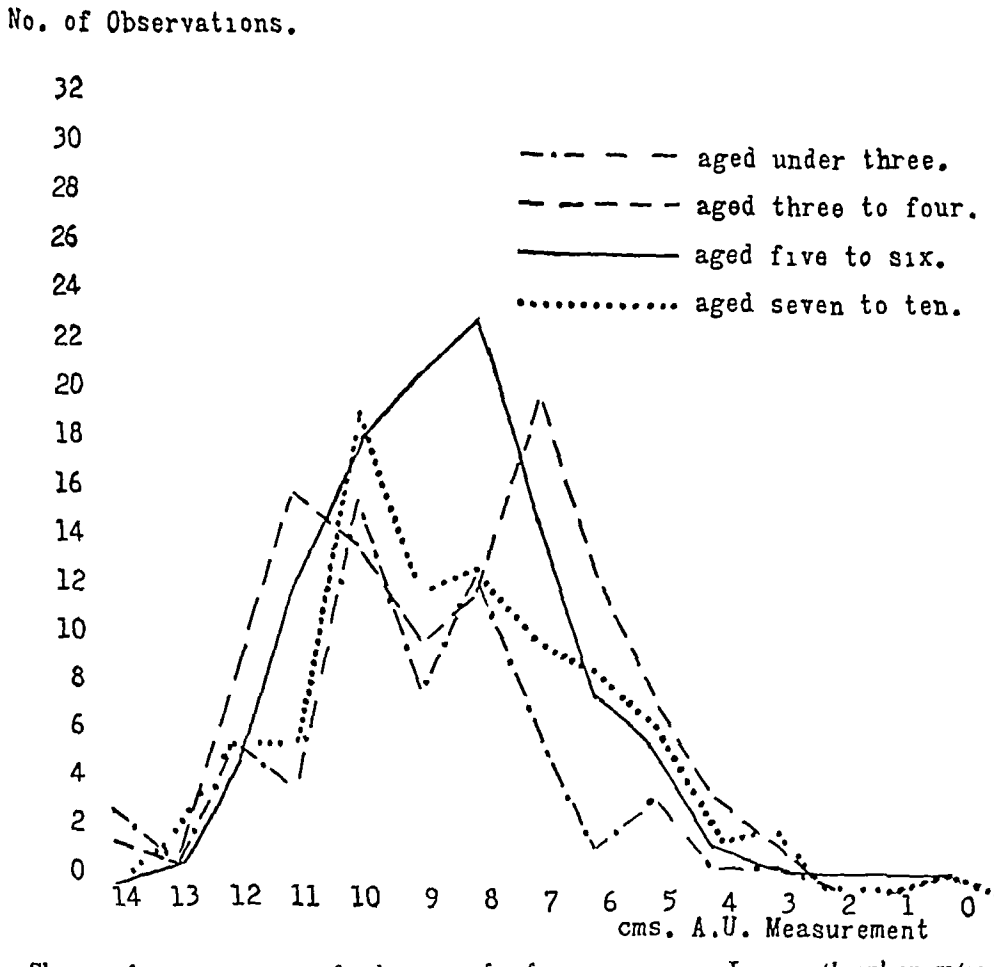
The initial gradual increase in the spleen rate may be ascribed to the gradually increasing chances of the child having received a severe infection with malaria, as it grows older, and the subsequent decrease to the development of immunity, which is acquired at an earlier age in the more malarious lines than in those with low spleen rates

(2) *Size of the spleen*—The frequent occurrence of two modes in a frequency curve of the spleen size has been noted by Christophers (1929), who interpreted it as meaning that there were two distinct sizes of spleen, a small one associated with acute malaria, and a larger spleen associated with the stage of immune infestation In the most malarious lines examined this bimodal curve was well marked, and some suggestion of bimodality was present in all the lines When this frequency curve is analysed according to the ages of the children examined, however, the bimodal curve is seen to be mainly an attribute of certain ages only The curves are shown in Graphs 1 to 4, in which the following attributes will be noticed —

(1) Series over 75 per cent, Graph 1 In the earlier ages, under three years, the curve is bimodal with the main mode at 10 cm from the umbilicus, and a smaller one at 8 cm from the umbilicus At three and four years the mode indicating the larger spleens has become more marked, and is at 7 cm from the umbilicus At five and six years this mode is the only one, though

the curve shows a marked skew to the left, which indicates the presence of a number of the smaller spleens which constituted the former mode at 10 cm From the age of seven onwards the mode at 10 cm is again the more important one, though the former chief mode at 7 cm is still visible

GRAPH 1

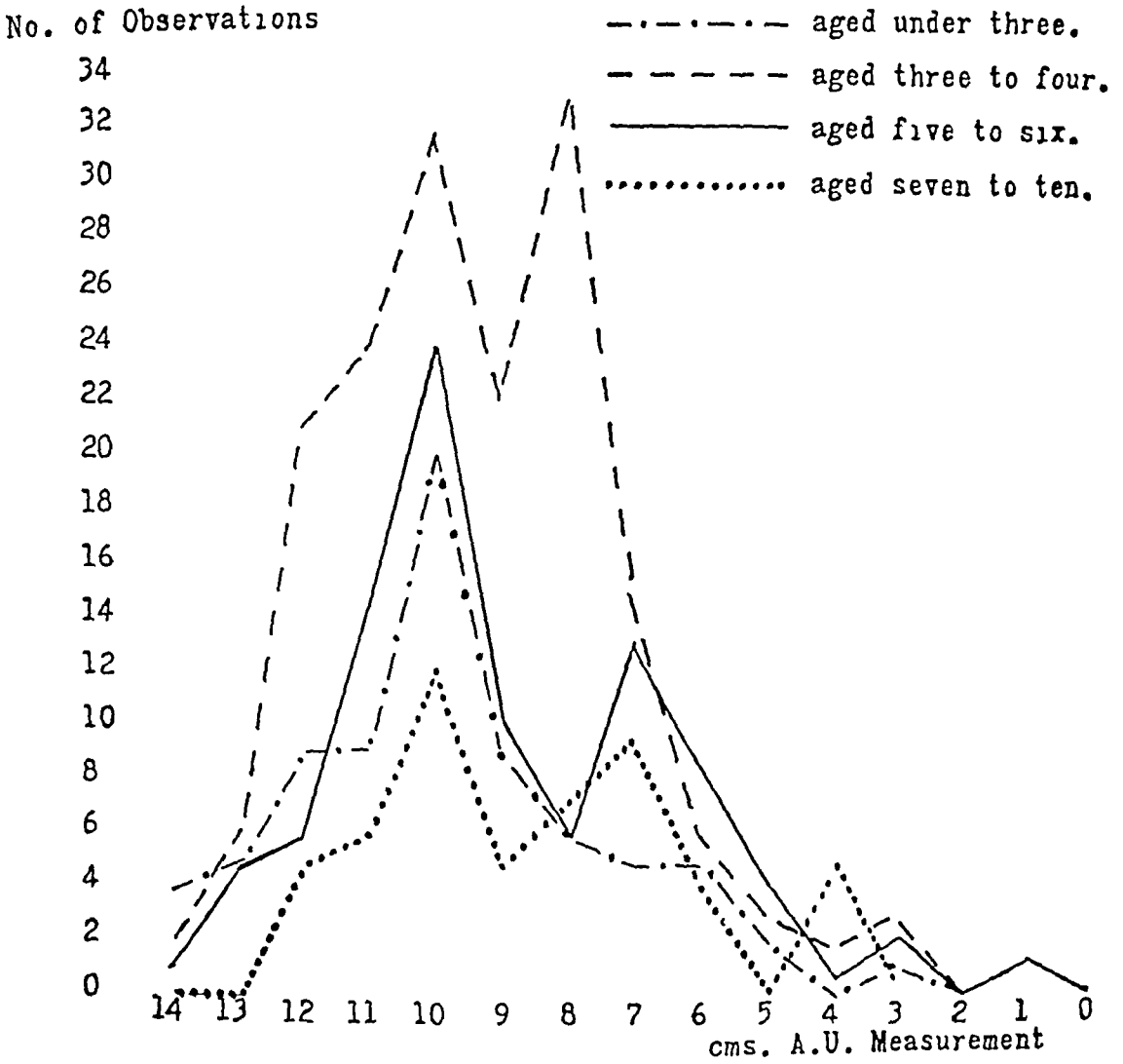


Showing frequency curves of spleen size for four age groups, Lines with spleen rates over 75 per cent

(2) Series 50 to 75 per cent, Graph 2 In the children under three years old there is a unimodel curve, the mode being at 10 cm from the umbilicus At three and four years of age the curve is definitely bimodal with apices at 10 and 8 cm, the two modes being approximately equal in size In the age

period five to six and seven to ten the curve is still bimodal, but the apex at 10 cm is considerably the more important one again

GRAPH 2

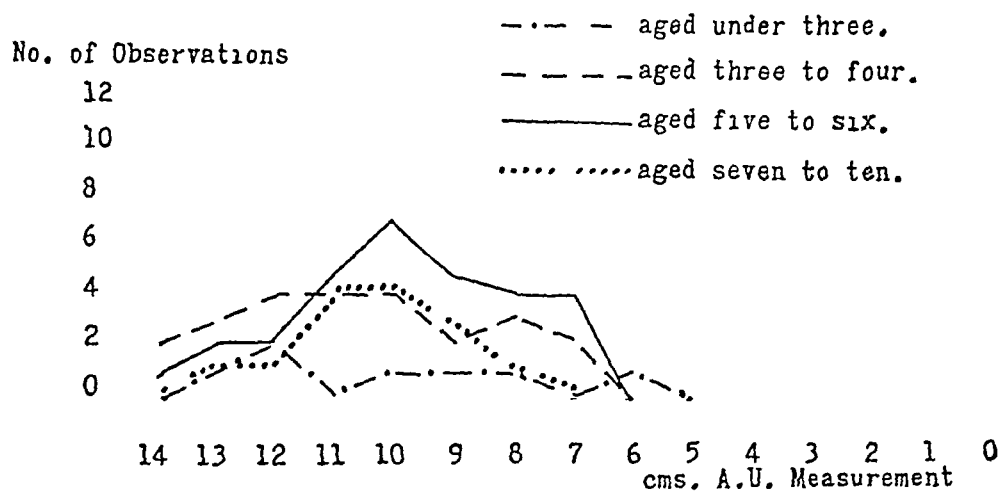


Showing frequency curves of spleen size for four age groups Lines with spleen rates between 50 and 75 per cent

(3) Series 25 to 50 per cent, Graph 3 Frequency curves for the different ages in this area are not reliable on account of the small numbers of enlarged spleens seen in each age group There appears to be no marked mode in the younger children, there is a suggestion of bimodality in the children

aged three to four and five to six, and certainly the larger spleens are more common at these ages, and after seven years the curve is once again unimodal

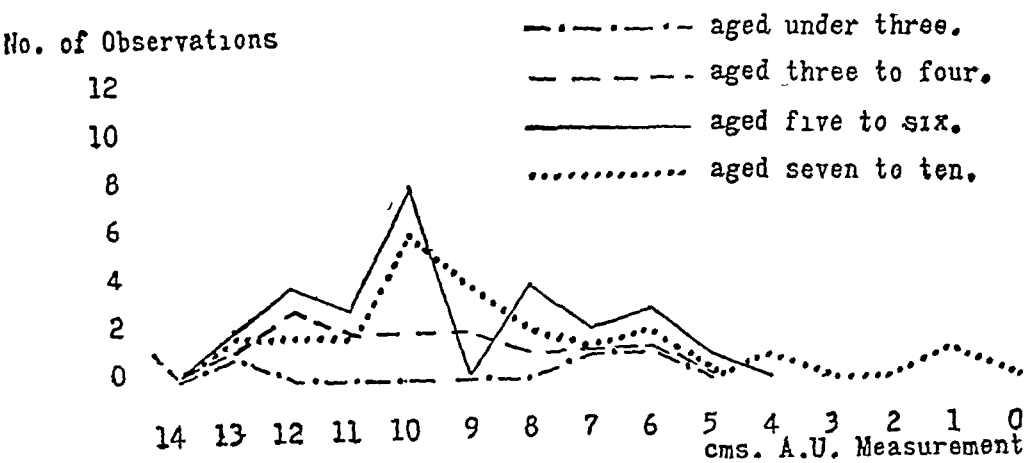
GRAPH 3



Showing frequency curves of spleen size for four age groups Lines with spleen rates between 25 and 50 per cent

(4) Series under 25 per cent, Graph 4 The findings here are similar to those seen in the previous series, but on account of the small numbers of enlarged spleens seen in each age period they are not reliable

GRAPH 4



Showing frequency curves of spleen size for four age groups Lines with spleen rates under 25 per cent

These findings are in accord with those of Christophers (1924), and the relative absence of large spleens in the earlier years agrees with the findings of Covell (1927) in the Andamans, that a community recently subjected to

the influence of malaria showed small spleens only The interpretation to be placed on these findings will be discussed at a later stage

The relation between the spleen size and the presence of parasites

Table XIII shows for each series the number of spleens of each size in which the blood was examined, and in which it was found positive

TABLE XIII

Showing the parasite infestations found in association with the different sizes of spleen

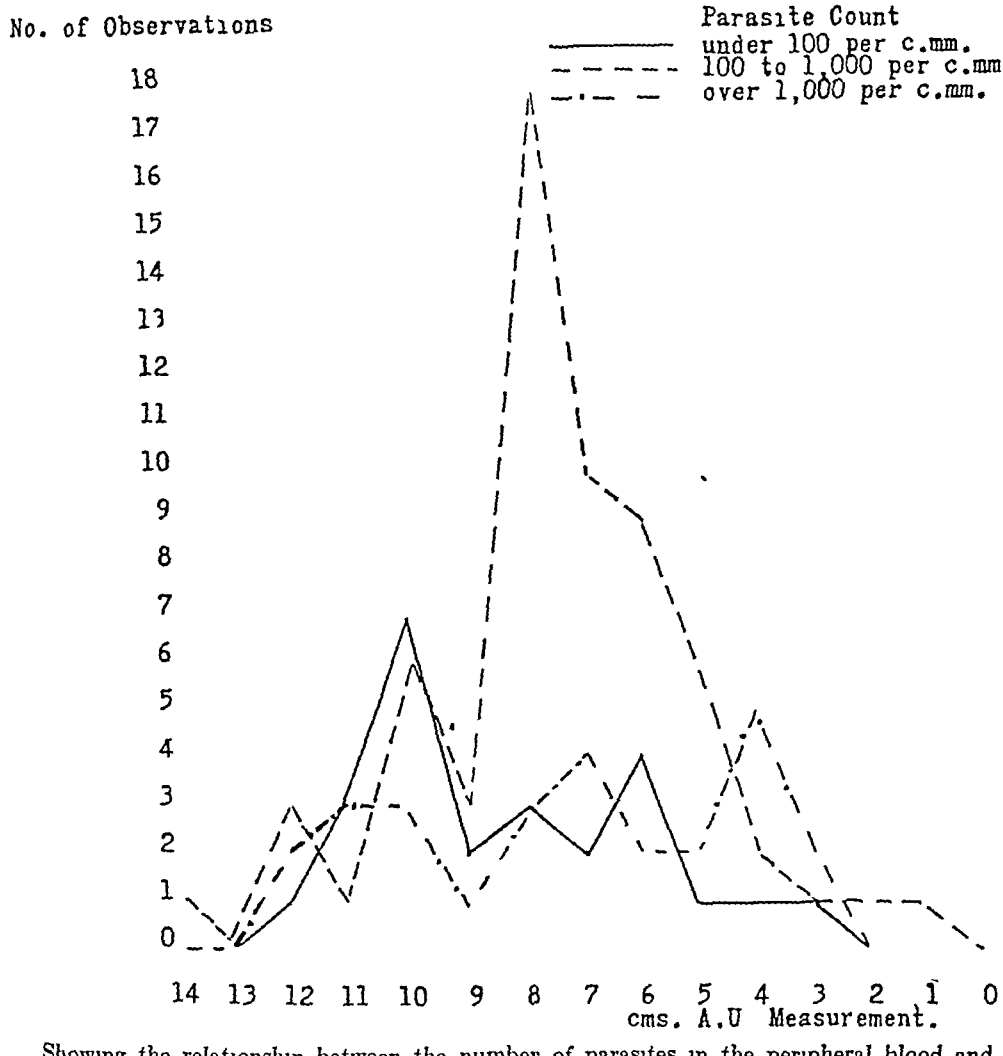
Apex umbilicus measurement	OVER 75 PER CENT		50–75 PER CENT		25–50 PER CENT		UNDER 25 PER CENT	
	Number examined	Number positive	Number examined	Number positive	Number examined	Number positive	Number examined	Number positive
Negative	41	22	100	44	58	18	82	10
14 cm	1	1	3	2	3	1	0	0
13 „	0	0	7	3	2	0	1	0
12 „	11	6	15	10	4	2	3	1
11 „	14	7	22	11	9	5	2	2
10 „	32	16	43	21	8	4	2	0
9 „	22	6	27	13	9	6	2	1
8 „	35	24	18	10	5	5	3	3
7 „	29	16	16	8	2	1	2	2
6 „	22	15	12	6	2	2	3	2
5 „	15	9	3	2	0	0	1	1
4 „	12	8	4	2	0	0	1	0
3 „	4	4	2	1	0	0	0	0
2 „	1	1	0	0	0	0	0	0
1 „	1	1	0	0	0	0	0	0
0 „	1	0	0	0	0	0	0	0
—2 „	1	0	0	0	0	0	0	0
—6 „	1	0	0	0	0	0	0	0

The parasite rate is higher amongst those whose spleens project to within 8 cm of the umbilicus, or less, than amongst those with smaller spleens in all the series except the 50–75 per cent series, in which case the parasite rate is the same in both cases Table XIII is epitomized in Table XIV below to bring this fact into prominence

TABLE XIV

Series	Spleen size					
	9–14 cm A U			8 cm A U or less		
	Number examined	Number positive	Percentage positive	Number examined	Number positive	Percentage positive
Over 75	80	36	48	122	78	63
50–75	117	63	54	55	29	53
25–50	35	18	51	9	8	89
Under 25	10	4	40	10	8	80
TOTAL	242	121	50	196	123	63

GRAPH 5

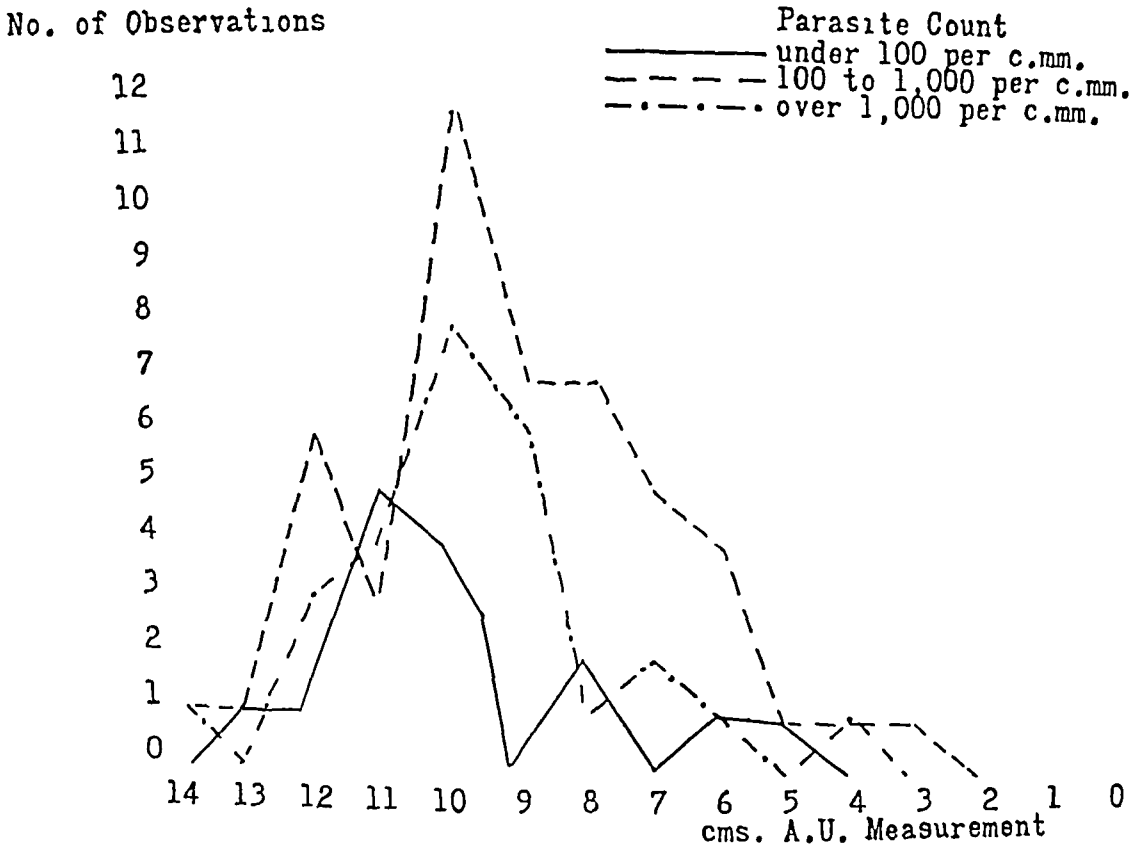


Showing the relationship between the number of parasites in the peripheral blood and the size of the spleen Lines with spleen rates over 75 per cent

Relation between the spleen size and the parasite count

In order to show this relationship the positive films have been divided into three categories, those with under 100 parasites per c mm, those with 100 to 1,000 per c mm, and those with over 1,000 per c mm, and frequency curves have been drawn in Graphs 5, 6 and 7 showing the number of each category in association with each size of the spleen in the different areas The figures for the two series with the lower spleen rates have been combined as the small number of positive bloods seen here makes complicated subdivision

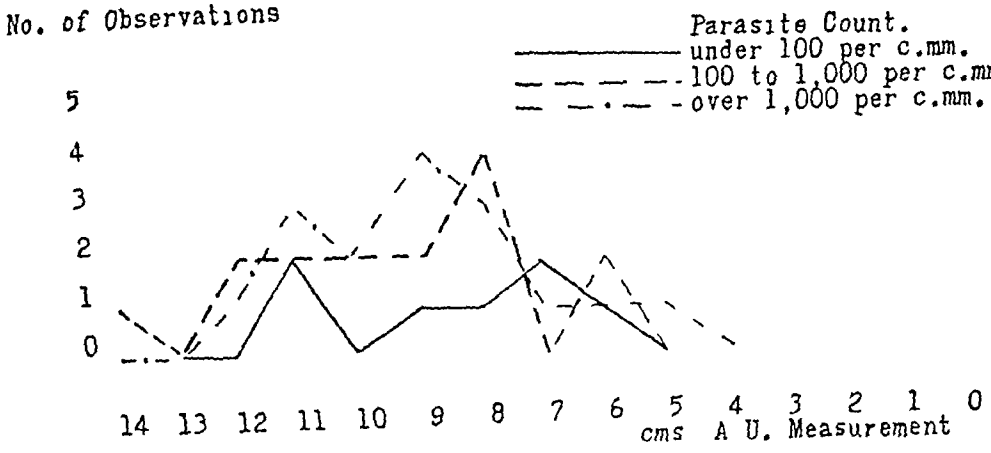
GRAPH 6



Showing the relationship between the number of parasites in the peripheral blood and the size of the spleen Lines with spleen rates between 50 and 75 per cent

unreliable In each of the other two series the low parasite counts are chiefly found in association with the smaller spleens projecting to within about 10 to 11 cm of the umbilicus, the moderate infections show a marked association with a larger size of spleen than the low infections, the heavy infections, with parasites counts over 1,000 per c mm, show no distinct preference for any particular size of spleen in the most malarious lines, while in the 50 to 75 per cent series they are definitely associated with an intermediate size of spleen

GRAPH 7



Showing the relationship between the number of parasites in the peripheral blood and the size of the spleen Lines with spleen rates under 50 per cent

Summary

(1) Lines with spleen rate over 75 per cent In these the aggregate spleen rate was 83 per cent, the parasite rate was 56 per cent The spleen rate reaches its maximum at three to four years of age and thereafter gradually declines A spleen frequency curve for all ages of children is bimodal, but analysis shows that the bimodal character is chiefly evident in children aged two to four, when there are two main sizes of spleen present, that reaching to within 10 cm of the umbilicus, and another reaching to within 8 cm In the older children aged seven to ten the smaller size of spleen is usually seen

The parasite count gradually diminishes throughout the whole ten year age period examined In those children under three years of age high parasite counts are the rule, while after this lower counts are more common

It was more usual to find positive blood films in those with large spleens projecting to within 8 cm of the umbilicus than in those with smaller spleens It was more usual for the moderate infections between 100 and 1,000 per c mm to be associated with the large spleens, the low parasite infestations were found together with the smaller spleens, while the very high parasite counts showed no preference for any particular size of spleen

(2) Lines with spleen rates between 50 and 75 per cent In these the aggregate spleen rate was 63 per cent and the parasite rate 50 per cent The spleen rate reached its highest at three to four years of age, and thereafter declined Before the age of three the modal size of spleen is small, at 10 cms from the umbilicus, after this there is a bimodal curve with apices at 8 and 10 cm in the children aged three to four After the age of four the larger spleens readily decline in importance

The parasite rate shows a gradual diminution throughout the whole age period examined Owing to the small number of very young children examined

the expected marked preponderance of heavy infections in the younger children was not seen

Positive blood films were found equally in association with spleens of all sizes, though it was again noted that the moderate parasite infestations occurred in association with a larger size of spleen than the very slight infestation, while the heavy infestations, with parasite counts over 1,000 per c mm occurred in association with an intermediate size of spleen

(3) Lines with spleen rates between 25 and 50 per cent The aggregate spleen rate in these lines was 43 per cent and the parasite rate was 43 per cent also The spleen rate reached its highest at five to six years of age, a slight decline being seen in the older children The spleen frequency curve for children of all ages shows a suggestion of bimodality in a marked skew to the right, but the small number of children with enlarged spleens seen in each age group makes accurate deductions from age analyses unreliable

The parasite rate appears to be at its maximum at five to six years of age, while high parasite counts were relatively more common in older children than was the case in more malarious lines

Positive blood films were more commonly found in association with the large spleens projecting to within 8 cm or less of the umbilicus The association between the size of the spleen and the intensity of infection is difficult to trace on account of the small numbers in each group when the figures are split up

(4) Lines with spleen rates below 25 per cent The aggregate spleen rate was 19 per cent and the parasite rate was 21 per cent The spleen rate appeared to be at its highest in the older children aged seven or eight There is here again a suggestion of bimodality in the spleen frequency curve for all ages but the paucity of the figures makes age analysis impossible

The parasite rate was at its highest at five to six years of age, but the figures being small it is impossible to say at what age the highest infestations more commonly occur

Positive blood films were more common in association with larger spleens than with the smaller ones, but the association between the size of the spleen and numbers of parasites is difficult to trace

Discussion

In all four series here described the same process of infection followed by immunity is going on, as is shown by the fact that, as far as they can be deciphered, the same changes in the spleen rate, spleen size, parasite rate, parasite count, etc, are taking place in all of them, differing only in degree and in the ages at which the changes take place

The process of infection in children in endemic areas has been variously regarded by different observers Some regard the children as suffering throughout childhood from a series of illnesses, each associated with the appearance of parasites in the peripheral blood and possibly enlargement of

the spleen, from which the child may recover, with disappearance of its symptoms though possibly without complete sterilization, to succumb to another attack at a later time, as a result of immunity developed during the initial attack later attacks may be less severe This might be termed the *discrete infection theory*

The second theory as to the mechanism of infection and immunity, applicable to hyperendemic areas, was propounded by Christophers (1924) and may be described as the *continuous infection theory* According to this all children in the area are constantly suffering from malaria, and all suffer equally, passing through continuous stages of acute infestation in early childhood, followed by immune infestation as they grow older This implies that the child has a practically continuous illness with only daily variations in its symptoms and signs such as the number of parasites in the peripheral blood, due to chance, and a gradual improvement due to the development of immunity, whilst it excludes the possibility of the disappearance of symptoms due to recovery from the disease

In trying to explain the facts seen in these series by one of these theories, there are difficulties encountered in applying the continuous infection theory, although it has been demonstrated as applicable in certain intensely malarious areas investigated by Christophers If all the children at any one age are equally infected and suffer equally, at any rate in the two hyperendemic series, then variations found in any series of children of one age must be explained The differences that are seen between the individual children in any one age group are (1) presence of parasites, some having parasites in the peripheral blood and others being free from them, (2) the varying number of parasites, high counts being encountered in some and low ones in others, though as a rule one type of count predominates, (3) presence of enlarged spleen, some having splenic enlargement and others not, and (4) size of spleen, in some age groups there being a definitely bimodal frequency curve The first two of these variations are easily explained on the grounds of daily variations due to chance, and errors of observation due to our known defective methods of examination, in favour of which explanation is the usual marked predominance of one type of parasite count The third variation is less easily explained, the spleen is not an organ which undergoes marked daily variations in size, it normally taking weeks at least for a moderately large spleen to become impalpable in an untreated person, so the presence of children without palpable spleens cannot be explained by chance daily variations, but must be ascribed either to the existence of uninfected children, or to some children having, at any rate partially, recovered from the disease The first of these explanations seems unreasonable, because, to take an instance, in the 50–75 per cent series, the ideas that 24 per cent of children aged three to four have escaped the effect of exposure to their environment, and that the infection amongst the others is sufficiently intense to keep up a condition of continuous infection, are mutually antagonistic, the second explanation is itself repugnant to the

continuous infection theory The fourth variation in the findings amongst any group of children of one age group, the existence of spleens of different sizes, is the most difficult to reconcile with the continuous infection theory If all the children in any one age group were suffering equally from malaria, then they should all have spleens of approximately equal size, centring round a single mode in the form of the normal frequency curve, the existence of a bimodal curve at any one age period is evidence that there are at least two stages of infection present in those children

All the phenomena here seen can, however, be explained without the slightest difficulty if we accept the following theories —

(1) The theory outlined in the first part of this paper of the relation between the spleen and parasite rates, which implies acceptance of the discrete infection theory, these account for the differing values of the spleen and parasite rate, and for the difference seen between the individuals in any series of children of the same age,

(2) Christophers' original splen theory to account for the differences in the size of the spleen, and

(3) A development of immunity, such as has long been recognized, to account for the changes taking place in the different age groups

In the first part of this paper a relationship has been shown between the spleen and parasite rates which can only be the result of the frequency of infective bites and the symptoms displayed could not exist if each bite did not produce its own definite train of symptoms, which implies acceptance of the discrete infection theory

If we admit the possibility that each individual undergoes a series of illnesses, recovering partially or completely between infections, then any series of children of the same age will include a number who are in the initial acute stage of attack, a number who have passed this stage and are recovering, and some who have recovered, the number in each of these conditions will depend on the frequency of infection in the area, the length of time for which the children have been exposed to it, that is, their age, and the degree of immunity developed as the result of previous attacks

The *splen* theory, with the modification stipulated above that only some 50 per cent of infections are of sufficient severity to give rise to enlargement of the spleen, readily explains the variations seen in the size of the spleen This theory said that a single infection with malaria produced a certain definite mean enlargement of the spleen, termed one *splen,* and that a double infection produced a greater enlargement termed two *splens,* etc A frequency curve of the sizes of spleen seen should therefore, in highly malarious areas, be definitely bimodal, those with a single infection centring round a mode at about 10 cm from the umbilicus, and those with more than one infection centring round a mode nearer to the umbilicus The double infections, which would produce the bimodal curve, should be rarer in the younger children in whom the spleen rate is low, and should be most common in those age groups

in which the spleen rate is at its highest, as a slight increase in the spleen rate is accompanied by a proportionately greater increase in the number of those with two *splens* Reference to Graphs 1 to 4 and Table XII will show that this is the case in those lines in which sufficient cases were secured for reliable analysis

The variations in the parasite findings amongst a number of children of the same age is explained by the different stage of attack in which they will be, in the case of younger children, who will be completely non-immune, a majority will show high parasite counts while a few who are recovering will show low counts, among the older children who have developed a certain degree of immunity medium and low counts will be the rule

The relation between the spleen size and the parasite count is explained on the following grounds, the high parasite counts occur almost entirely amongst the young children who are presumably undergoing their first infection, and they therefore usually occur in connection with the spleens of one *splen* size The children rapidly acquire, probably after their first infection, a sufficient degree of immunity to protect them from these high parasite infestations, as is shown by the early age at which they become relatively less common A large spleen being, *ex hypothesi* associated with at least two infections, and occurring most frequently in children who are two or more years old, is seen in those who have had time to develop some slight immunity and do not show the very high parasite counts, hence the medium parasite count is found most frequently in connection with a large spleen The very low parasite count is most common amongst the older children who are partially immune and are capable of resisting a number of infections, consequently in them double infections are less common and these low parasite counts are associated with the smaller spleens

The immunity developing among these children appears to do so on the following lines, before the initial attack they are completely non-immune and the first attack is associated with a high parasite count After this they develop some resistance to the parasite, so that in future attacks a lower parasite count is seen, but they have not yet developed sufficient immunity to ward off an attack completely, and as the children grow older and their chance of escaping infection therefore becomes less, a greater number become infected After having been repeatedly infected they become more resistant, very low parasite counts are seen after infection, and they gradually acquire a sufficient degree of immunity to ward off a number of the infections to which they are subjected These processes may be seen at work in (1) the rapid diminution, from the earliest ages, in the numbers of parasites seen, (2) the slower diminution in the parasite rate, the diminution in which is probably partly due to the smaller numbers of parasites present in the films and the consequent increased difficulty in finding them (3) The gradual increase in the spleen rate as the chances of infection increase, followed by a decrease as sufficient immunity is developed to ward off infection (4) The decrease in the number of larger

spleens seen in the older children, due again to the ability to resist infection, thus diminishing the number of double infections

An immediate objection that arises to the discrete infection theory in the more malarious lines is that an untreated case of malaria is a lengthy affair, and that the frequency of infection ought to be so great in such areas that it is ensured that every child is repeatedly re-infected before it has had time to recover from its initial attack It is not disputed that this does occur in some areas, and where it does so the frequency of attacks in the individual becomes so great that they merge into one another and a state of continuous infection arises, but it is thought that it is only in a few intensely malarious areas that this does occur, and not in all areas with spleen rates over 50 per cent There is no evidence that in the majority of highly endemic areas infection is of very frequent occurrence, while there is considerable circumstantial evidence that it is not, and attention may be drawn to the following points (1) the rarity with which gametocytes are found in such children as those examined here, out of 714 children examined, of whom 250 were infected with *P falciparum*, only 10 had more than 12 crescents per c mm and thus were capable of transmitting infection, (2) the comparative rarity of the carrier species of anophelines In this survey they were difficult to find and only represented 14 per cent of the total catch inside houses, (3) the low sporozoite rate met with even in the chief carrier species, Ramsay (1930) has dissected very large numbers of anopheline mosquitoes in Assam over a period of three years, and of the chief carrier species, *A minimus* found only 0 7 per cent infected in the glands, the greatest percentage found infected at any time of year, being 1 7 per cent in the month of June, lastly, (4) we have the evidence of Blacklock and Gordon (1925), working in West Africa in an endemic area with a spleen rate of 56 per cent, who investigated the age distribution of infections amongst children under two years of age These observers found that the parasite rate in these young children rose very gradually, increasing by not more than five per cent in any month, and that it was not until the age of eighteen months that it reached its subsequent steady level of 42 per cent

All these facts point to the possibility of a high spleen rate, and hyper-endemic conditions, occurring in an area where the frequency of inoculation with malaria is not great, occurring at sufficient intervals to allow the children to recover, at any rate partially, between attacks, and having a series of discontinuous illnesses, the intervals between which depend on the frequency of inoculation with sporozoites

Summary

This paper is founded on the results of nine series of blood and spleen examinations made during a Malaria Survey in Assam and in the Sir A L Jones Laboratory, Sierra Leone

An explanation is put forward of the relationship commonly seen between the spleen and parasite rates in endemic areas Briefly this is that (1) only

some 60 per cent of infections are discovered by our present methods of examination, (2) the parasite rate amongst those with enlarged spleens remains practically constant in most endemic areas, whatever the spleen rate, at about 60 per cent, and this is a useful criterion of the percentage of infections being discovered, (3) a 'true infection rate' can be calculated from the parasite rate by multiplying it by a factor based on the parasite rate amongst those with enlarged spleens, (4) this 'true infection rate' and the spleen rate show a relationship to each other which can only be explained on the grounds that only some 50 per cent of infections are of sufficient severity to produce enlargement of the spleen

The blood and spleen findings in the Assam series are analysed and the following points brought out (1) The parasite rate reaches its height in the first two years of life, except in the less malarious lines, and thereafter declines, (2) the numerical frequency of parasites in the blood of those infected decreases rapidly after the first two years, (3) the spleen rate reaches a maximum in children aged three to six, the exact age depending on the severity of the malaria in the lines, and thereafter declines, (4) a frequency curve of the size of spleen shows definite characteristics in different age groups, large spleens being more common in children aged three to six, (5) a large spleen is more frequently associated with a positive blood than a small spleen, (6) a large spleen is generally associated with a moderate parasite infestation of 100 to 1,000 parasites per c mm, small spleens being usually associated with either a very small or a very high parasite count

The conclusions drawn from the facts observed are (1) that infection in these children is discrete rather than continuous, re-infection occurring normally at long intervals, although the observations were made in a hyperendemic area, (2) that the *splen* theory adequately explains the changes in the size of the spleen, and (3) that a relative immunity to malaria is gradually acquired by the children living in these lines

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TRANSMISSION OF INDIAN KALA-AZAR BY THE BITE OF *PHLEBOTOMUS ARGENTIPES*

BY

LIEUT.-COLONEL H E SHORTT, F.Z.S., I.M.S.,

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AND

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(*Kala-azar Commission*)

[Received for publication, February 27, 1931]

OF the numerous attempts made in the past to bring about transmission of Indian kala-azar by the bite of *Phlebotomus argentipes* all have failed, whether these experiments were on animals or on human volunteers. The reason for these failures is still to seek but we are now able to record the first successful transmission by the bite of this insect.

The known life-history of *Leishmania donovani* in *P. argentipes* pointed in no uncertain fashion to the probability that the bite of this insect was the method by which *Leishmania* infection was conveyed to a new host and the experiment to be described below gives confirmation to this view.

The methods of feeding the flies on cases of kala-azar and subsequently of feeding the infected flies on Chinese hamsters were precisely those detailed in previous publications of the Kala-azar Commission and need no further mention. Details of the series of experiments forming the subject of this communication are given in the accompanying table.

TABLE

Showing result of transmission experiments

Serial number of hamster	Date of commencement of experiment	Total number of feeds by *P argentipes*	Number of flies known to be not infected	Number of flies known to be infected	Number of flies condition not known	Date of termination of experiment	REMARKS	RESULTS
1	18-8-29	22	0	2	20	4-9-29 Found dead	No proper examination possible	
1 (*a*)	10-9-29	149	14	49	86	5-1-31 Found dead	No proper examination possible	
2	19-8-29	132	6	31	95	15-5-30 Found dead	Spleen slightly enlarged	Microscopic examination negative Cultures contaminated
3	22-8-29	45	0	15	30	18-8-30 Found dead	No proper examination possible	
4	25-8-29	108	0	33	75	20-7-30 Found dead	No proper examination possible	
5	10-9-29	144	6	38	100	4-2-31	Sacrificed Spleen not enlarged	Microscopic and cultural results positive
6	11-9-29	143	5	37	101	4-2-31	Sacrificed Spleen not enlarged	Microscopic and cultural results negative

Remarks

The only detail in which this experiment differed from all previous ones was in the somewhat longer period elapsing between the commencement of the experiment and its termination by post-mortem of the animal This period was one of about seventeen months—511 days to be exact—whereas the longest period of any previous similar experiment on hamsters was 435 days We do not think this an important point since the hamster showed no macroscopic enlargement of the spleen, a fact indicating the probability of a comparatively recent infection, i e , an infection occurring late in the series of feeding experiments

We think the fact that only one out of 42 hamsters experimented on along similar lines became infected indicates that the infection rate by the bite of *P argentipes* may be a low one This would be one possible explanation of the slow spread of kala-azar in normal inter-epidemic periods and of its more rapid advance during an epidemic, when the number of infective kala-azar cases has increased to a maximum, resulting in a high percentage of infected flies It is possible also, that the more rapid succession of passages of the parasite from man to fly and vice versa during an epidemic would enhance its virulence to a degree quite out of proportion to that possessed by it during non-epidemic periods For this reason we believe that part of our previous lack of success in obtaining transmission was due to the fact that transmission experiments on a large scale with *P argentipes* and a really susceptible animal, such as the Chinese hamster or man, were only undertaken when the recent kala-azar epidemic was already on the wane and the virulence of *L donovani* was already lessening The corollary to these deductions is that for the confirmation on an adequate scale of our present successful experiment we may have to wait for the outbreak of the next epidemic in Assam

Acknowledgments

We wish to express here our great indebtedness to all members of the staff of the Kala-azar Commission whose combined efforts have enabled us, after years of work, to obtain a successful transmission In this connection we would especially mention Sub-Assistant Surgeons Chiranji Lal and Sribas Das, on whose shoulders the bulk of the routine microscopic work has rested, Field Assistant James John, whose survey work resulted in a continuous stream of kala-azar cases suitable for our experiments, U Kun Mohon Roy, senior Laboratory Assistant, who was responsible for all details in connection with experimental animals, and senior Insect Collector Nur Mohammed, who was indefatigable in the laborious routine of our entomological work

frequently dissected and the buccal armature was found to correspond fairly closely with that described by Sinton (1928) for *P babu* var *nigri*, also from time to time unfed specimens from the same batch of flies were mounted for identification and no other species was identified

Annandale (1910) described *P babu* from Calcutta, but later (1910*a*, 1911 and 1911*a*) he decided that it was identical with *P minutus* Rond Sinton (1928) now claims, after re-examination of Annandale's original type specimens, that *P babu* is a distinct species and different from *P minutus* Rond which he further claims has not been found on this side of India These kaleidoscopic changes in entomological nomenclature are not uncommon but are nevertheless very confusing We should perhaps have been on safer ground had we referred to these flies as being of the 'minutus group' instead of as *P minutus* (*babu*) though criticism of our paper on this account would amount to quibbling

Our object was to throw light on the feeding habits of certain sandflies, particularly *P argentipes*, and not to upset biological preconceptions Had the latter been our object we should have been more careful not to offend the susceptibilities of the systematic entomologist by the use of somewhat loose nomenclature However, the present writer was aware that sandflies of the minutus group (*P minutus* Rond in particular) had been reported previously only as feeders on lizard blood, therefore when in one batch of flies he found that 5 out of 6 were reported to contain human blood he sent them to Major J A Sinton, I M S, for confirmation regarding their identity Major Sinton reported that 4 out of 5 specimens were *P babu* var *nigri* Although these were not the actual specimens whose blood-meal had been identified, they were sandflies caught in the same place at the same time, and from naked-eye and grosser microscopical appearances they were identical The very strong presumption is, therefore, that they were also *P babu* var *nigri* Subsequently we did not feel that it was necessary to obtain confirmation of our identification It is thus probable that the large majority of the flies reported in our paper as containing human or bovine blood which were classified as *P minutus* (*babu*) were specimens of the species now described by Sinton (1928) as *P babu*, or *P babu* var *nigri*, and it is beyond the bounds of possibility that some of them were not of this species

With regard to the technique employed in the precipitin tests, the flies were dissected on mica cover-slips, which had been 'flamed' previously to destroy all organic matter, by means of dissecting needles which had been similarly 'flamed' If the gut contents were adherent to the cover-slip this was pared down and the small portion of cover-slip with the adherent blood was dropped into a numbered test-tube If the gut contents was solid it was rolled into the test-tube from the cover-slip The clean* test-tubes were supplied by

*The tubes supplied by Col Lloyd had been prepared for use by an elaborate purification process so arranged as to yield tubes which are dry, sterile and absolutely free from organic matter, as employed in his department as a routine

Col Lloyd and the subsequent identification was carried out by him With very few exceptions the specimens after being dissolved were divided into two portions, one was tested against anti-human and the other against anti-bovine high titre precipitin serum, in the exceptional cases the material was considered insufficient for the double test and was tested against anti-human serum only The results were recorded as ++, +, ± or — If the results was ++ or +, it was taken as conclusive evidence that the blood of the species from whose blood the antiserum had been made was present, a negative result meant that it was *not detectable* The ± sign was seldom employed and when the results were analysed it was treated as a negative result

About the technique of the actual precipitin test, the present writer does not propose to make any comment The specificity of this test is universally accepted and when it is carried out under the immediate direction of an officer of the experience of Col Lloyd any discussion on the reliability of the results would amount to an impertinence

A few controls were carried out with flies fed on known blood but, except in as far as the time factor was concerned, it was thought entirely unnecessary to include them in the paper

Col Shortt's failure, in the past as well as in the series now reported, to persuade sandflies of the minutus group to feed on man in captivity does not, in the present writer's opinion, justify his criticism of the work of other observers The result of the simple experiment which he carried out is exactly in accordance with the experience of the present writer, but his conclusion is totally unjustifiable It has never been claimed that the horse was a total abstainer from water just because, when he was taken home after the occasion of his proverbial refusal to drink, he finished his bran mash

The present writer is entirely satisfied that the results reported in the paper written by Col Lloyd and himself are absolutely accurate in as far as the identification of all the blood-meals and of the sandflies of the species *P argentipes* and *P papatasii* are concerned He is also convinced that the results show that the sandflies of the minutus group common in Calcutta, *P babu* according to the most recent entomological opinion, frequently feed on the blood of man and of cattle, and that the conclusions, which were based —as is perfectly obvious from the title of the paper—on laboratory feedings only, with regard to the feeding habits of the flies of this species were substantially correct

In order to get confirmation regarding the identity of the actual specimens of the sandflies of the minutus group whose blood-meals have been tested, the present writer decided to carry out a few further tests with flies of this group sending the head and thorax of the dissected flies to Major Sinton for identification In each batch some undissected specimens caught in the same place at the same time were included Col Lloyd being at present on leave Rai Bahadur Mitra, Officiating Imperial Serologist, kindly consented to carry out

the precipitin tests As was the case in the previous series the specimens when sent to the serological department were numbered and the worker carrying out the serological test had no knowledge of the source of the specimen A few controls are also included in this short series

The results of the precipitin tests are shown in the table

TABLE

Batch	Serial numbers	Precipitin test result		Source of the flies	Identity of the flies
		Ruminant	Human		
A	1–3	—	—	Cow-shed in Vaccine Depôt	
B	4–8	—	—	Cow-shed in Village	
C	9	+	++	Cow-shed in Vaccine Depôt	One identified as *P shortti* Two identified as *P babu* var *niger* Identified by Major Sinton
	10	+	+		
	11	+	—		
D	12	++	—	Do	All as *P babu* var *niger*
	13	+	—		
	14	++	—		
	15	++	—		
E	16–19	—	—	Do	
	20	++	—		
	21	—	—		
	22	+	—		
	23	—	—		
F	24–33	—	++	Laboratory-bred flies fed on a patient	*P argentipes*
G	34–43	—	—	Laboratory-bred flies fed on a mouse	*P argentipes*

Any extensive comment on the results would be superfluous

Only one batch was sent to Major Sinton as it was a crucial one and the results of his identification satisfied any doubts we might have had He identified the three dissected flies as *P babu* (2 ♀ ♀) and *P shortti* (1 ♀) (*vide* Table) My entomological assistant, Mr S Mukerji, identified the others, using Major Sinton's drawings as an additional guide

Thus, all the flies were of the minutus group, being either *P shortti* or *P babu* var *nigei* Specimens of each species contained ruminant blood and of the latter species, at any rate, human blood

The control flies which had been fed on human blood gave uniformly positive and uniformly negative results with anti-human and anti-bovine serum, respectively, and those fed on a mouse, uniformly negative results with both sera

My thanks are due to Major J A Sinton, VC, IMS, for actual help in the identification of sandflies used in this series of experiments and for many detailed letters on the subject of the identity of sandflies of the minutus group, to Rai Bahadur G C Mitra, Officiating Imperial Serologist, for identifying the blood-meals, and to my entomological assistant, Mr S Mukerji, for much practical assistance

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CORRESPONDENCE—THE NATURE OF 'BLACK SPORES'

We have received the following communication from Colonel S L Brug
—ED

'THE HAGUE, 10*th January*, 1931

DEAR SIR,

On reading the paper of Bruce Mayne entitled "The nature of black spores, associated with the malarial parasite in the mosquito and their relationship to the tracheal system" which appeared in this *Journal* in 1929 in Vol XVII on p 109, I was struck by some quotations from my paper "Die schwarzen Sporen ('black spores') bei der Malariainfektion im Muckenkorper" (*Arch f Protistenkunde*, 1916, XXXVI, p 188) which are obviously erroneous In literature there is a tendency to perpetuate erroneous quotations and therefore I might put matters right, be it somewhat late, by putting side by side my original statements and Bruce Mayne's quotations which apparently refer to them

Arch f Prot, XXXVI

Page 190 Ein ganzer Muckenmagen mit schwarzen Sporen, deren Lage notiert war, wurde 24 Stunden bei 37° in 10 per cent KOH belassen Die Sporen blassen etwas ab, die schwarzen werden gelbbraun, aber sie losen sich nicht
Nach 2 Monaten (bei Zimmertemperatur) sind die Sporen noch etwas blasser geworden, aber keine hat sich gelost

Page 190 Ruge gibt an, dass die schwarzen Sporen in physiologischer Kochsalzlosung anderthalb Jahre und langer unverandert bleiben

Page 194 Denkbar ist, dass das Sprengen oder Einreissen der Cysten den Reiz bildet zur Chitinisierung der Cysten oder deren Inhalt, ebenso wie die Sprengung der Teilungsformen in Menschenkorper den Fieberanfall (bei Malaria tertiana und quartana) veranlasst Page 197 Das Einreissen der Cystenwand bildet fur die Mücken den Reiz zur Chitinisierung des Cysteninhalts

Ind Jour Med Res, XVII

Page 114 Tested by immersion in a solution of 10 per cent of potassium hydroxide at body temperature for periods of 1 to 60 days, he found that they bleached only slightly and were not dissolved

Page 114 Brug states that Ruge kept these bodies in salt solution for about 6 months and they remained unaltered

Page 115 Brug makes the unusual suggestion that the oocyst ruptures as a result of this chitinization of its contents, a process which he considers of a similar nature to the rupturing of the schizonts of the malaria parasites in the body of man during a paroxysm in a case of tertian or quartan fever

* * * * * *

I am,
Yours very truly,
(Sd) S L BRUG'

CORRESPONDENCE—FEEDING HABITS OF *PHLEBOTOMUS MINUTUS*

We have received the following from Lieut-Colonel H E Shortt, I M S
—ED

'PASTEUR INSTITUTE OF INDIA,
Kasauli, Punjab, 28th March, 1931

SIR,

It has been brought to my notice that certain expressions used in my paper entitled "Note on the Feeding Habits of *Phlebotomus minutus*" appearing in the January (1931) number of the *Indian Journal of Medical Research* might conceivably be construed as casting doubt on the general reliability of the precipitin test Needless to say no such intention existed and, in this letter, I hasten to take the opportunity to remove any such misconception

In my paper I gave the logical position which the very puzzling results referred to seemed to bring about but there are other explanations one would wish to explore very thoroughly before throwing doubt on the universally acknowledged reliability of the precipitin test

It is unnecessary for me to add that I have every confidence that the methods employed by Col Lloyd, whom I have known for many years, are the most reliable to be found in serological technique

Yours faithfully,
(Sd) H E SHORTT,
Lieut-Colonel, I M S'

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